STANDARDIZATION AND PHARMACOLOGICAL SCREENING OF *GANDHAGA SARKKARAI*

The dissertation Submitted by

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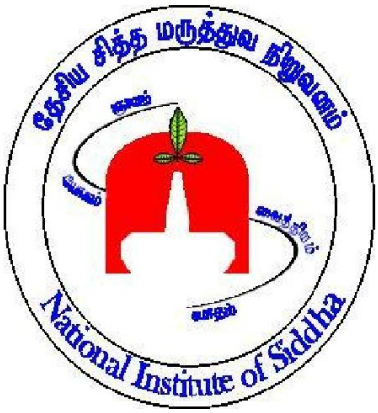

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CONTENTS

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1.Introduction

The basic purpose of any science is to bring solutions to human problems . All sciences since even the most ancient times as a tradition , have been developed to freeing the human race from Obstacles. Obstacles such as disease ; discomfort of inhospitable environments , drastic weather conditions and other conditions .

According to the ancient picture , Science dealt with crucial and fundamental human problems – the prevention of death , the extension of lifespan , and above all, the spiritual advancement of humanity.

The Siddha system of medicine, one of the ancient system speaks a numerous remedies solving basic human problems using everything available in the world for that purpose – every plant , animal or mineral or metal. The medicinal knowledge remains un-supposed in the entire world. Based on this vision the compassionate Siddha revealed arts and sciences of Yoga , medicine , astrology ,occultism , astronomy , vaastu saastra , Alchemy .

The Siddha Tradition , identified with the tamil speaking land , remains a mystery to practitioners of other native medicinal system of India; not to speak of those involved in the study of traditional systems of medicine.

The term 'Siddha' comes from the word "Chit" meaning 'consciousness that illumines'.So the correct way of saying Siddhar is Chittar the one who abides as consciousness .Any system of tradition is more of an evaluation rather than an overnight invention.

"That which cures physical ailments is medicine

That which cures physcological ailments is medicine

That which prevents ailments is medicine

That which bestows immortatality is medicine".

Siddhar Thirumoolar .

The Traditional Tamil Siddha System has existed from time immemorial , even before written history . It is very difficulty to say exactly when it originated . Evidently, it confines

the system to that area only for instance , Siddha traditional system of medicine, we covers and consider expansive possibilities , several diseases and ailments generated from many varying climatic condition and changes .

Siddha system of medicine is a science which treats body and mind. Life style advocacies, selection of functional foods and person oriented treatment regimen are the uniqueness of Siddha system of medicine.

Siddha system of medicine is an integrated part of Indian system, which is a very potent and unique system in existence and practiced in India for thousands of years and above. It is an earliest medical science that stress on positive health, a harmonious blending of physical, mental, social, moral and spiritual welfare of individuals. The Siddha system has developed a rich treasure of medicinal knowledge that includes the use of herbs, metals and minerals. It is a traditional system of medicine which is gradually evolved along with the Dravidian's culture and hence this system is also known as Dravidian system of medicine. Siddha system also deals with the concept of salvation in life. The aim of Siddha medicine is to make the body perfect, imperishable and to promote longevity.

Siddha system of medicine is considered the oldest documented medicine system of the world. It evolved in south India and the knowledge of siddha medicine was completely flourished in the period of Indus Valley Civilization.

The exponents of Siddha system of medicine are called Siddhars. They are the super human beings with high culture and intellectual abilities. It is considered that Siddha medicine was created by Lord Siva and he is the first Siddhar. There were 18 important Siddhars in olden days and they developed this system of medicine. Hence, it is called Siddha medicine. Siddhars were spiritual adepts who possessed the Ashta siddhis or the eight supernatural powers. They practiced intense yogic practices, including years of periodic fasting and meditation and were believed to have achieved supernatural powers and gained the supreme wisdom and overall immortality. Through this spiritually attained supreme knowledge, they wrote scriptures on all aspects of life, from arts to science and truth of life to miracle cure for diseases.

Food habits and daily activities of an individual play a major role in causing disease. The physical functions of the body is mediated and maintained by three vital forces. They are

Vali, Azal and *Iyam*. In normal state they are called three forces or Muthathu that sustain and nourish the body. In disease state when the three forces are vitiated they are called Mukkutram. When the three forces are in balance one is healthy. When vitiated singly or combination bring about disease. Emotion and stress also stimulates the *Udal thathukal* (7 physical constitution) ending up in a disease.

The structural aspect of the human body is said to be "*Udal Thathukkal* " (i.e. the physical component of the human body) which consists of seven elements: first is *Saaram* (Plasma) responsible for growth, development and nourishment; second is *Senneer* (Blood) responsible for nourishing muscles, imparting colour and improving intellect; the third is *Oon* (Muscle) responsible for shape of the body; fourth is *Kollzuppu* (Fatty tissue) responsible for oil balance and lubricating joints; fifth is *Enbu* (Bone) responsible for body structure and posture and movement; sixth is *Moolai* (Nerve) responsible for strength and the last is *Sukilam* (Semen) responsible for reproduction.

The functional units of the human body are said to be "*Uyir Thathukkal*" (i.e. *Vatham, Pitham* and *Kabham*). They are considered as three pillars of health and support, the structure and functions of the body. They are involved in regulating all the functions of the body and maintain the balance in the physical, emotional and mental spheres. These Uyir thathukkal co-exist in all the cells of the body. They function in a harmonious manner to create a balance. The factors assumed to affect this equilibrium are environment, climatic conditions, diet, physical activities and stress. The food, which is the basic building material for the human body, gets processed into these body tissues, humors and waste products to determine the balance of the Uyir thathukkal in the body.

Since, this system was bestowed to as at a time when science was not developed, Siddhars were designed the treatment according to the above mentioned parameters[2]. Siddhars have classified the disease into 4448 types. Megam is one of the sexually transmitted disease caused by Bacterium Trepenoma palladium ,which it has 4 stages of signs and symptoms. Syphilis has been known as the Great imitator .

Syphilis is a sexually transmitted infection caused by the bacterium Treponema palladium. The signs and symptoms are varying in which four stages (primary , secondary ,

latent , tertiary).The primary stage with a single chancre. In secondary stage rashes occurs on hand and soles of the feet.In latent there will be no symptoms. In tertiary syphilis there are Gummas , neurological ,or heart symptoms.

In 2015 , about 45.4 million people were infected with syphilis , with 6 million new cases.During 2015 , it caused about 107,000 deaths , down from 202,000 in 1990. After decreasing dramatically with the availability of penicillin in 1940s, rates of infection have increased in many countries. In 2015 , Cuba became the first country in the world to eliminate mother-to-child transmission of Syphilis.

The Siddha system of medicine uses a fascinating combination of herbs, minerals and metals to promote good health and longevity. Before preparing medicines, Siddhars laid a great emphasis in purification of raw drugs. More than 80% of the Siddha medicines are formulated by herbal products, but in certain life threatening disease and in many chronic diseases, Siddhars enumerated some herbo-metal, herbo-mineral and metalo-mineral formulations.

Gandhaga Sarkkarai is one of the herbo-mineral formulation mentioned in ancient Siddha literature text "*Anuboga Vaithiya Navaneetham*", part -VI, page no:46,47. preparation no:793.

When traditional literatures were reviewed, it revealed that Gandhagam has anti-bacterial and histaminic properties, Karisalai has anti-inflammatory and analgesic properties and the research articles revealed that the individual ingredients of *Gandhaga sarkkari* possess Anti-histamine, Anti-inflammatory and Analgesic activity but as a finished product no pharmacological activities has been carried out for this formulation.

Many research workers have conducted a number of pharmacological and toxicological experiments for various Siddha formulations which revealed that the toxicity of the crude drug is quite different from that of the finished Siddha formulations. Hence the researcher selected the drug *Gandhaga Sarkkarai* to standardize and evaluate the pharmacological activities such as Anti-histamine activity, Anti- inflammatory activity and Analgesic activity.

2. Aim and objectives

Aim

To Standardization and evaluate the Pharmacological profile of the test drug "***Gandhaga Sarkkarai''*** in animal models.

Objectives

The following methodology was adopted to Standardization and evaluate the Pharmacological profile of the test drug.

- Review of various information (*Siddha* and Modern) relevant to the study.
- Preparation of the drug as per classical *Siddha* literature.
- Analytical study of the prepared drug
    - Physicochemical and phytochemical analysis
    - Chemical analysis to evaluate acidic and basic radicals.
    - Elemental analysis
    - Scanned Electron Emission with EDAX
    - Fourier Transform Infrared
    - X- Ray Diffraction
    - Thermo-gravimetric analysis
    - HPTLC

Evaluation of pharmacological activities in animal models.

- Anti-histamine activity - Evan's dye method
- Analgesic activity - Eddy's Hot plate method
- Anti-inflammatory activity - Carrageenan induced paw oedema method.

3. Materials and methods

Standard Operating Procedure of *Gandhaga sarkkarai*

Drug selection

The drug ***Gandhaga Sarkkarai***, is a Herbo-mineral *preparation (Sarkkarai*= one of the *32* types of *internal* medicine), mentioned in *Siddha* text "***Anuboga Vaithiya Navaneetham***", (part -VI, page no:46,47. preparation no:793) indicated for *Megam (syphilis), Premegham , Kiranthi , Purai (Whole Abscess) , Kai kaal kudaichal (Joint pain).*

Ingredients

1. Purified *Gandhgam (Sulphur)* - *280 gms*
2. Karisalai *(Eclipta alba)* - *250 ml*
3. Vellai vengayam *(Allium cepa)* - *250 ml*

Collection of the Plant materials

Gandhagam was purchased from a well reputed country shop in Parrys, Chennai. *Karisalai and Vellai vengayam* were freshly collected from Tambaram sanatorium, Tamilnadu.

Gandhagam was purified and the medicine was prepared in the *Gunapadam* laboratory of National Institute of Siddha.

Identification and Authentication of the drug

1. Mineral drug was authenticated by Dr.M.Suresh Gandhi, Department of Geology, University of Madras, Chennai.
2. The Herbal drugs were identified and authenticated by Dr .D. Aravind M.D(s), Botanist, National Institute of Siddha, Tambaram Sanatorium, Chennai.

Purification process

Karisalai - Eclipta prostrata ,*Linn* (Whole plant)

Washed it in RO water

Vellai vengayam - Allium cepa , *Linn* (Tuber)

Washed it in RO water

Gandhagam (Sulphur) **:**

The *kalkam* of *Lowsonia inermis* was mixed in cow's curd and placed in a mud pot. The mouth of the pot was covered with a cloth. Sulphur was placed over the cloth. The pot was covered with another suitable pot and buried in the ground. The entire setup was subjected to *pudam* with five dung cakes. The sulphur which melts and settles down was collected. This procedure was repeated for 7 times .

Before Pudam on First Day of Purification

Figure no -1

Method of Preparation :

Purified *Ganthagam* was grounded with juice of Eclipta *prostrata* (Karisalai) juice from morning 6 a.m. to 12 noon after that it was dried in sun light. This procedure was repeated for 3 days. The same procedure was repeated with juice of Allium *cepa* (vellai vengayam). Finally the mixture was grounded until it reached the consistency of fine powder.

Drug storage :

The trial drug *Gandhaga Sarkkarai* was stored in clean, dry wide mouthed glass bottles.

Labelling

Name of the preparation	:	*Gandhaga sarkkarai*
Date of preparation	:	30/10/2017
Dose	:	1varagan (4.2g), bd
Adjuvant/Vehicle	:	Honey
Indications	:	*Megam , Premegam , Kiranthi , Purai*
Date of expiry	:	30/10/2018
Colour	:	Yellowish brown
Reference	:	Anuboga Vaithiya Navaneetham,

Therapeutic administration of drug

Form of medicine	-	*Chooranam*
Route of administration	-	Oral
Dose	-	4.2gm twice daily
Vehicle	-	Honey

INGREDIENTS OF *GANDHAGA SARKKARAI*

Gandhagam

Before purification

Figure no – 2

During purification

Figure no - 3

After purification

Figure no - 4

ECLIPTA ALBA – KARISALAI

Figure no – 5

ALLIUM CEPA - VELLAI VENGAYAM

Figure no - 6

Gandhaga Sarkkarai preparation

Grinding with *karisalai* leaf juice

Figure no – 7

Figure no - 8

4.1 Botanical review

Botanical Review of *Eclipta prostata – Karisalai*

Synonyms:

Common name as "Bringaraj".

Taxonomical classification:

Kingdom : Plantae

Phylum : Angiosperms

Order : Asterales

Family : Asteraceae

Genus : Eclipta

Species : prostata

Parts used : Whole Plant.

Karisalai is a prostrate herb with ascending branches to 70 cm tall in height.

Leaves : Laneolate and oblong.

Flowers : Terminal or Axillary heterogamous heads, white in colour.

Fruit : Achene.

Habitat **:** Warmer parts of the World.

Phytography :

Its about 25-100 cm long.

Heads are Sub-globose , about 1.25 cm broad.

It grows peak in the month of August to Febrauary.

Microscopic Description :

Cells of both upper and lower epidermis are polygonal and wavy in outline and are transversed by antisocytic and anomocytic types of stomato which are more in numbe on lower side . The cells of lower epidermis are more wavy in outline.Transverse section of the root circular in outline with a central narrow xylem surrounded by a layer of cambium , phloem and a wide zone of cortex interspersed by the circles of giant air spaces.The outer most tissue is composed of ill developed 1-2 layered brownish cork cells, which often exfoliated leaving behind the inner secondary cortical zone.

Chemical constituents:

Alkaloids, cardiac glycosides, Polypeptides, Thiophene derivatives, Steroids, Triterpenes & Flavanoids .

Common Uses of Eclipta prostrta :

The Whole plant is astringent , depurative , emetic , ophthalmic , purgative , styptic and tonic.

It is used in the treatment of dropsy , and liver diseases , anaemia , diphtheria etc,,..

Externally it is used as an oil to treat Hair loss and is also used to applied on eczema , dermatitits , wounds .

The juice of the plant is mixed with aromatic oils , is used in jaundice and catarrhal problems.

The leaves are used in the treatment of Scorpion stings.

The roots are emetic and purgative.

A Black colour dye is obtained from the plant. It is used as hair dye.

Leaves are used to treat Epilepsy in India.

Scientific reviews of Eclipta prostrata :

Analgesic Activities seen in Eclipta alba:

Experimental evaluation of the Analgesic activity of Eclipta prostrata (L) Hassk.

Total alcoholic extracts of Eclipta prostrata was undertaken to study the analgesic activity in albino mice and albino rats by using different standard experimental models.

Pandey et al.(1997) reported that an alcohlic extract of plant (200 mg/kg) showed analgesic effect in rats due to the coumarin compounds.

Analgesic Studies on total alkaloids and alcohol extracts of Eclipta prostrta.

The present experimental research work was undertaken to determine the Analgesic activity of the total extract of Eclipta prostrata and isolated alkaloids of eclipta in albino mice using standard experimental methods as the Tail-clip method .

Further studies done by Sawant et al. (2004) confirmed that the alkalloids and alcohlic extracts of E. alba contain variety of natural pain killing substances which help to overcome mild to moderate pain without any side effect.

Anti-inflammatory Activities seen in Eclipta alba :

Evaluation of Anti-inflammatory activity of Eclipta prostrata in rats.

The Anti-inflammatory effect of the plant of Eclipta prostrata was using Carageenin , mediators such as histamine and serotonin induced paw oedema and cotton pellet induced granuloma tests for their effect on acute and chronic inflammation models in rats.

Anti-inflammatory activity of methanolic extract of Eclipta prostrata.

The methanolic extract of leaves of Eclipta prostrata was investigated for Anti-inflammatory activity in wistar albino rats with carrageenan induced paw oedema method .

Evaluation of Anti-inflammatory activity of roots of Eclipta prostrata .

The aim of the study was to evaluate the anti-inflammatory activity of methanolic extract of root part of Eclipta prostrata in rats.

Pharmacognostical, phytochemical and analgesicactivity of *Eclipta prostrata :*

Further Extracts were screened for analgesic activity by using Eddy's hot plate and heat immersion methods , at a dose level of 50 &100 mg/kg body weight .Ethyl acetate extract showed a dose dependent ans significance (p-.01) analgesic activity in all the tested methods compared to diclofenac treated mice . Methanolic extract also exhibited analgesic activity .

Anti-inflammatory , analgesic , anti-oxidant activity of herb *Eclipta prostrata* :

The present study was designed to investigate the anti-inflammatory , analgesic , anti-oxidant activity of the methanolic extrtact along with its organic soluble fractions of the herb Eclipta prostrata.

Botanical review of *Allium cepa – Onion*

Taxonomical classification:

Kingdom	:	Plantae
Phylum	:	Angiosperms
Class	:	Monocot
Order	:	Asparagales
Family	:	Alliaceae
Genus	:	Allium
Species	:	cepa

Habitat :

It is a herbaceous bulbous plant, with a biennial seed production , annual herb production , annual bulb production , and the bulb being the edible part.

It is developed with ramified and superficial roots.

Tubular waxy leaves , which are dark green on arieal part ,

At the base – Tunic – They are thin , wrap over , protective on the outside , while they are meaty on the inside.

The floral stalk is rigid , hollow , and waxy and the plant is grown over a metre tall with an umbrella inflorescence and in spherical in shape .

Flowers are hermaphrodite , white .

Fruit – Occurs in Capsule.

Actions :

Diuretic,

Anti inflammatory,

Analgesic,

Antirheumatic,

Antibiotic.

Chemical constituents and uses:

There are more than 100 sulfer compounds in onion that have anti-inflammatory effects. However, many are known to change readily when heated or are broken down after cutting. As with garlic, onions also contain the volatile, natural antibiotic oil called ALLICIN, responcible for its pungent flavour.the tear evoking lachrymatory chemical released when onion is crushed or cut is **thiopropanal S-oxide**, turns into sulphuric acid when it comes into contact with water.

Phytochemicals of Allium cepa:

Onion contains quercetin, aflavonoid (one category of anti oxidant compounds), and allicin. The flavonoid quercetin, an anti oxidant found in onions, helps eliminate free radicals in the body, inhibits low density lipoprotein oxidation, protects and regenerates vitamin E, and helps to circumvent the harmful effects of heavy metal ions. Recent studies at Wageningen Agricultural University, the Netherlands, showed thwt the absorption of quercetin from onion is twice that form tea and more than three times that from apples. In addition, onions are very rich in chromium, a trace mineral that helps cells respond to insulin, Vitamin C and numerous flavonoids.

Action and Medical Uses :

Onions posses properties allied to those of Garlic, but in a milder degree, and the absorption in its oils and influence upon the system is somewhat similar to that of the oil of garlic.

A cataplasm of onions pounded with vinegar , applied for anumber of days and changed 3 times a day, has been found to cure Corns.

Onions are useful in hemorroids, dysentry, flatulence, dyspesia , jaundice, spleenopathy, vomiting, malarial fever etc.

Scientific reviews of *Allium cepa* :

Evaluation of analgesic and anti-inflammatory effects of fresh onion juice in experimental animals :

Onion is a well known traditional medicinal plant that has been consumed for its putative nutritionaland health benefits for centuries. This study was carried out to d etermine the possible analgesic and anti –inflammatory activity in experimental animals .

Analgesic activity studies with a polyherbal formulation containing plant Allium cepa

This study reveals that Analgesic activity ,which activity MEEAC at the dose of 400 mg/kg was higher than aspirin, where MEEAC maybe potential source to obtain pain in rats.

4.2 Mineralogical review

MINERALOGICAL ASPECT OF SULPHUR

Sulfur or sulphur is a chemical element with symbol S and atomic number 16. It is abundant, multivalent, and non-metallic. Under normal conditions sulfur atoms form cyclic octatomic molecules with a chemical formula S_8. Elemental sulfur is a bright yellow crystalline solid at room temperature. Chemically, sulfur reacts with all elements except for gold, platinum, iridium, tellurium, and the noble gases Name : Sulphur .

Symbol	: S
Atomic Number	: 16
Group	: 16
Period	: 3
Block	: p
Element category	: Non-metal
Standard atomic weight	: 32.065(5)g/mol
Electoronic configuration	: (Ne) $3s_2,3p_4$
Density	: 2.07g/cm^3
Melting Point	: 338.36K
Boiling point	: 717.8 K
Heat of fusion	: 1.727 KJ/mol
Heat of Vapourisation	: 45 KJ/mol

Atomic Properties :

Oxidatiive states	: 6,5,4,3,2,1,-1,-2 (Strongly acidic oxide)
Electronegativity	: 2.58 (Pauling Scale)
Ionization energy	: 999.6 KJ/mol

Miscellaneous :

Crystal structure	: Orthorombic
Magnecity	: Diamagnetic
Thermal Conductivity	: .205 w/m/K
Natural forms	: Pure element
	Sulphide
	Sulfate.

Terminology :

In Bible, Burning Sulphur is called as '**Brimstone**'.

Characteristics:

Sulphur melts to a blood red liquid and emits a blue flame due to sulphur-dioxide which is best observed in the dark.

The strong smell of the sulphur refers to the odor of Hydrogen sulphide, which is the principal odor of untreated sewage.

Sulphur is insoluble in water , but soluble in Carbon- disulphide, to a lesser extent in benzene and tolouene.

Crystalline form : Rhombic, monoclinic form

Allotropes	: 30
Isotopes	: 25

Occurrence :

Sulphur is found near the hot spring and valcanoic region, pacific ring of fire. Currently mined valconoic deposits are Indonesia, China and Japan. Sicily is famous for Sulphur mines.

Natural compounds of Sulphur:

Pyrite (Iron Sulphide)

Cinnabar (Mercury Sulphide)

Galena (Lead Sulphide)

Sphalerite (Zinc Sulphide)

Extraction Process:

By third century , Chinese discovered that sulphur could be extracrted from the pyrite.

Silician process&Frasch process : Sulphur is obtained by Frasch process as 99.5% purest form.

Chemically obtained by Claus process from hydrogen sulphide.

Physical properties of sulphur:

Character	:	Crystalline in character
Smell	:	Faint smell
Taste	:	No taste
Solubility	:	In soluble in water
Soluble	:	Carbon sulphide sparingly in alcohol & ether
Melting point	:	Low melting point.

Physio-Chemical Properties :

Sulphur is a multivalent, non metal, abundant, tasteless & odorless , is yellow and crystalline solid .

In nature it is pure element, (or) sulphide, sulphite minerals

The smell of the sulphur compare to rotten egg smell, the presence odour because of hydrogen sulphide (H_2S)**.**

General uses of sulphur :

Sulphur is commercially important in manufacture of chemical such as sulphuric acid the chemicals is also used for manufacture of sulpha drugs. In agriculture, the sulphur is the fore most important crop nutritive element & it is also used as a fertilizers it is also used to manufacture poultry feeds. Sulphur is used in medicines only after it is refined well.

Medicinal uses:

Sulphur is used in scabies. The fumes of burning sulphur are said to cures gout and rheumatic affections. In organic sulphur reduces the motility and invasion of MDA-MB-231 human breast cancer cells. It is also used as the following purposes in medical uses such as,

Due to the development of resistance and availability of better antimicrobials, sulfonamides are not commonly used now expect in a few cases.

- Urinary tract infections: sulfonamides can be used in areas where resistance is not high.
- Nocardiosis: high doses of sulfonamides can be used.
- Toxoplasmosis: sulfonamides and pyrimithamine are the treatment of choice.
- Trachoma and conjunctivitis: tetracyclines are the drug of choice, sulfonamides are used as alternative.
- Malaria: Sulfadoxine is used with pyrimethamine in chloroquinone resistant malaria.
- Prophylactic use in patients allergic to penicillins, sulfonamides may be used for prophylaxis of streptococcal pharyngitis in rheumatic fever.
- Topical sulfadiazime are used in bacterial conjuctivitis; mafenide and silver sulfadiazine are in turn to prevent infection.
- Ulcerative colitis; sulfasalazine is useful in ulcerative colitis and rheumatoid arthritis.

4.3 Gunapadam review

Sulphur – Gandhagam – கந்தகம்.

There are 64 kinds of *Paashaanas* which are classified into two types

Natural (32)

Synthetic (32)

Sulphur is one of the *natural Paashaanas* which is bitter and astringent in taste.

Gandhagam is the element of *Theayu (FIRE).*

Other Names:

- Natham
- Paraiveeriyam
- Athitha Prakasam
- Beejam
- Chendurathathi
- Deviuram
- Ponvarni.

Types:

Based on the colour the *Gandhagam* has been divided into *four* types. They are,

White

Red

Golden Yellow

Black

Action:

- Laxative
- Antiseptic
- Tonic
- It also increases the bile fluids.
- It increases various body secretions
- When Sulphur is used in high doses it causes diarrhea

General characters of *Gandhagam*:

"நெல்லிக்காய்க் கந்திக்கு நீள்பதினெண் குட்டமந்தம்
வல்லை கவிசைகுன்ம வாயுகண்ணோய் - பொல்லா
விடக்கடிவன் மேகநோய் வீறுசுரம் பேதி
திடக்கிரசு ணீகபம்போந் தேர்."

This is considered to useful in the treatment of 18 types of *skin disease.*

Medicinal uses:

The medicinal uses of the Gandhagam (Sulphur) are various.

They are

- Liver Enlargement
- Abdominal distension
- Eye diseases
- Chronic venereal diseases
- Chronic diarrhoea
- Gastric ulcer
- Poisonous bites
- Fever
- Chronic dysentery .

Eclipta prostata – Karisalai - கரிசாலை

Synonym :

Kaiyanthagarai

Kaiyan

Thegarajam

Bringarajam

Karippan .

Types of Karisalai :

White , Yellow , Red , blue.

Whereas *White* type of *Karisalai* will be available in all places for our collection.

Parts Used : Whole plant.

Organoleptic Characters :

Taste	: Bitter
Character	: Heat
Division	: Acrid.

Actions :

Chologauge

Hepato-protective

Tonic

Alterative

Emetic

Purgative .

Medicinal Uses :

"குரற்கம்மற் காமாலை குட்டமோடு சோபை

யுரற்பாண்டு பன்னோ யொழிய – நிரற்சொன்ன

மெய்யாந் தகரையொத்த மீளி ண்ணு நற்புலத்துக்

கையாந் தகரையொத்துக் கால்" .

(அகத்தியர் குணவாகடம்)

- Karisalai is useful in treating Liver Diseases like Jaundice , Hepatitis A&B .
- It is useful in the treatment of throat infections .
- Karisalai chooranam is used to cure Anaemia with the combination of Aya chenduram.
- 90 drops of juice of Eclipta prostrata (leaves) is mixed with water or butter-milk to cure Snake-bites.
- Its useful in hair-growth activity.
- Extracts are used to cure Ear-ache as Ear drops.

Allium cepa – Vengayam – வெங்காயம்

Synonyms :

Ulli

Gayam

Sukkirantham

Nichhiyam

Palandu .

Parts used :

Vengayapoo

Kizhangu

Thaal

Seeds

Organoleptic properties :

Taste : Bitter

Character : Heat

Division : Acrid .

Actions :

Stimulant

Aphrodisiac

Demulcent

Expectorant

Diuretic , Rubefacient.

Medicinal Uses :

“ வெங்காய வித்து குன்மம் வீட்டும்”

- Seeds of *Allium cepa* will be very useful in curing the disease like *Gastric ulcer*.
- Seeds of *Allium* enhances the aphrodisiac activity.

“வெப்பமூ லங்கிரந்தி வீறுரத்த பித்தமுடன்
செப்புநா அக்கரந்தி ராத்தாகம் - வெப்புக்
கடுப்பறுமந் தஞ்சந்நி காசம்வயிற் றுப்பல்
தடிப்பேறும் வெங்காயத்தோல்” .

(அகத்தியர் குணவாகடம்)

- Tubers of onion will cure hemorroids , thirst , oral ulcers , hypertension , eczema like diseases.
- Mixture of onion with 2-3 seeds of pepper will useful in the treatment of fever.
- Onion juice is use as antidote for the tobacco toxicity .
- Onions posses properties allied to those of Garlic, but in a milder degree, and the absorption in its oils and influence upon the system is somewhat similar to that of the oil of garlic.

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5 . Analytical studies

Analytical study of the drug *Gandhaga Sarkkarai* brings the validation to be used as a medicine by subjecting the drug to many analysis and determining its quality and effectiveness. Analytical study includes many studies such as its *organoleptic properties, physicochemical properties* and *phytochemical properties* and also to assess the active principles and elements present in the drug. Thus analytical study brings the efficacy and potency of the drug. As per AYUSH protocol for analytical study, the following parameters were evaluated. Analytical study of the drug includes:

1) **Organoleptic characters**

   Colour, Odour, Texture, Taste

2) **Phytochemical analysis**

3) **Physicochemical analysis**

   Determination of Ash Values and Physical characterization

4) **Chemical analysis**

   Preliminary Basic and Acidic radical studies

5) **Elemental analysis**

   Inductively Coupled Plasma Optical Emissions Spectrometry (ICP- OES)

6) **Analysis of particle size**

   Scanned Electron Emission (SEM) with Energy Dispersive X-ray analysis (EDAX)

7) **Fourier transform infrared (FTIR)**

8) **X- ray Diffraction**

9) **Thermo gravimetric analysis (TGA)**

10) **HPTLC-Higher performance thin layer chromatography .**

5.1 Organoleptic characterization

The organoleptic characters of the sample drug *Gandhaga sarkkarai* were evaluated. 1gm of the test drug was taken and the following characters were seen.

- Colour
- Odour
- Texture
- Taste

Other morphology characters were viewed by naked eye under sunlight. Then the result were noted.

Colour

The medicine was taken into watch glasses and placed against white back ground in white tube light. It was observed for its colour by naked eye.

Odour

The medicine was smelled individually. The time interval among two smelling was kept 2 minutes to nullify the effect of previous smelling.

Taste

Small amount of *Gandhaga Sarkkarai* was kept over the tip of the tongue.

Results

The results of Organoleptic characters were showed in Table no - 1

5.2 Phytochemical analysis

The preliminary phytochemical screening test was carried out for each extracts of *Gandhaga Sarkkarai* as per the standard procedure. The Experimental Procedure was done at The T.N. Dr. M.G.R Medical university, Guindy, Chennai-32.

[1] Detection Of Alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

i) Mayer's Test

- Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide).
- Formation of a yellow colored precipitate indicates the presence of alkaloids.

ii) Wagner's Test

- Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide).
- Formation of brown / reddish precipitate indicates the presence of alkaloids.

iii) Dragendroff's Test

- Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide).
- Formation of red precipitate indicates the presence of alkaloids.

iv) Hager's Test

- Filtrates were treated with Hager's reagent (saturated picric acid solution).
- Presence of alkaloids confirmed by the formation of yellow colored precipitate.

[2] Detection Of Carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

i) Molisch's Test

- To 2 ml of plant sample extract, two drops of alcoholic solution of α- naphthol are added.
- The mixture is shaken well and few drops of concentrated sulphuric acid is added slowly along the sides of test tube.
- A violet ring indicates the presence of carbohydrates.

ii) Benedict's Test

Filtrates were treated with Benedict's reagent and heated gently.

- Orange red precipitate indicates the presence of reducing sugars.

[3] Detection Of Glycosides

Extracts were hydrolyzed with dil. HCl, and then subjected to test for glycosides.

i) Modified Borntrager's Test

- Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes.
- The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution.
- Formation of rose-pink color in the ammonical layer indicates the presence of anthranol glycosides.

ii) Cardiac glycoside (Keller-Killiani test)

- Extract was shaken with distilled water (5 mL). To this, glacial acetic acid (2 mL) containing a few drops of ferric chloride was added, followed byH2SO4 (1 mL) along the side of the test tube.
- The formation of brown ring at the interface gives positive indication for cardiac glycoside and a violet ring may appear below the brown ring

[4] Detection Of Saponins

i) Froth Test

- Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes.
- Formation of 1 cm layer of foam indicates the presence of saponins.

ii) Foam Test

- 0.5 gm of extract was shaken with 2 ml of water.
- If foam produced persists for ten minutes it indicates the presence of saponins.

[5] Detection Of Phytosterols

Salkowski's Test

- Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand.
- Appearance of golden yellow color indicates the presence of triterpenes.

[6] Detection Of Phenols

Ferric Chloride Test

- Extracts were treated with 3-4 drops of ferric chloride solution.
- Formation Of Bluish Black Color Indicates The Presence Of Phenols.

[7] Detection Of Tannins

Gelatin test

- The extract is dissolved in 5 ml of distilled water and 2 ml of 1% solution of Gelatin containing 10% NaCl is added to it.
- White precipitate indicates the presence of phenolic compounds.

[8] Detection Of Flavinoids

i) Alkaline Reagent Test

- Extracts were treated with few drops of sodium hydroxide solution.
- Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

ii) Lead acetate Test

- Extracts were treated with few drops of lead acetate solution.
- Formation of yellow color precipitate indicates the presence of flavonoids.

[9] Detection Of Proteins And Aminoacids

i) Xanthoproteic Test

- The extracts were treated with few drops of conc. Nitric acid.
- Formation of yellow color indicates the presence of proteins.

ii) Ninhydrin Test

- To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes.
- Formation of blue color indicates the presence of amino acid.

[10] Detection Of Diterpens

Copper Acetate Test

- Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution.
- Formation of emerald green color indicates the presence of diterpenes

[11] Gum And Mucilage

To 1ml of extract add 2.5ml of absolute alcohol and stirring constantly.

- Then the precipitate was dried in air and examine for its swelling properties.
- Swelling was observed that will indicate presence of gum and mucilage.

[12] Test For Fixed Oils And Fats

Spot test

- A small quantity of extract is pressed between two filter papers.
- Oil stain on the paper indicates the presence of fixed oils.

[13] Test For Quinones

Extract were treated with sodium hydroxide blue or red precipitate indicates the presence of Quinones.

Results

The results of phytochemical analysis were presented in Table no-2.

5.3 Physicochemical analysis

Physicochemical studies of the trial drug have been done according to the WHO guidelines. The physical properties of *Gandhaga Sarkkarai* was analyzed at The T.N. Dr. M.G.R Medical university, Guindy, Chennai-32.

Physicochemical studies of the drugs are necessary for standardization, as it helps in understanding the significance of physical and chemical properties of the substance being analyzed in terms of their observed activities and especially for the determination of their purity and quality. The analysis includes the determination of ash value, Loss on drying of the sample at 105ºC, pH value, Extractive value. These were carried out as per guidelines.

1. Determination of pH:

Five grams of *Gandhaga Sarkkarai* was weighed accurately and placed in clear 100 ml beaker. Then 50 ml of distilled water was added to it and dissolved well. After 30 minutes it was then applied in to pH meter at standard buffer solution of 4.0, 7.0, and 9.2. Repeated the test four times and average was recorded.

2. Loss on drying of the sample at 105ºC

4g of test drug *Gandhaga sarkkarai* was weighed in a previously weighed 100ml beaker and heated in an oven at 105ºC for 5hours. Cooled in a desiccator and weighed. Repeated the procedure till constant weight was obtained. The percentage loss in weight of the test drug was calculated by the following formula.

Calculation:

$$\text{Percentage of loss on drying at 105ºC} = \frac{\text{Loss in weight of test drug}}{\text{Weight of test drug taken}} \times 100$$

3. Ash content

Total ash content

4g of test drug *Gandhaga sarkkarai* was weighed accurately in a previously ignited and tared silica dish. The material was evenly spread and ignited in a muffle furnace at 600^0C until it became white indicating the absence of carbon. The dish was cooled in a desiccator and weighed. As carbon free ash cannot be obtained in this manner, the dish was cooled and the residue moistened with 2 sufficient quantity of water. Dried on a water bath and then ignited in the electric furnace to get the constant weight. Cooled the dish in a desiccator and then weighed. The percentage of total ash of air-dried materials was calculated as per the formula given below.

Calculation:

$$\text{Percentage of total ash} = \frac{\text{Weight of the ash}}{\text{Weight of test drug taken}} \times 100$$

4. Acid-insoluble ash

The total ash of the test drug *Gandhaga sarkkarai* was found out as described above. To the dish containing the total ash was added 45 ml of 1: 5 hydrochloric acid in three portions of 13 ml each time. Boiled gently for 5 minutes and filtered. Collected the insoluble matter on an ashless filter paper (Whatman No.41) and washed with distilled water until the residue was free from acid. Transfer the filter paper containing the insoluble mater to the original dish. Dried and ignited to the constant weight. Cooled the dish in a desiccator, and then weighed. Calculation was made by given formula.

Calculation:

$$\text{Percentage of acid-insoluble ash} = \frac{\text{Weight of the acid-insoluble residue}}{\text{Weight of test drug taken}} \times 100$$

5. Water soluble ash

Total ash 1g was boiled for 5min with 25ml water and insoluble matter collected on an ash less filter paper was washed with hot water and ignited for 15 min at a temperature not exceeding 450^0C in a muffle furnace. The amount of soluble ash is determined by drying the filtrate.

6.Water-soluble extractive of the test drug

4 g of the test drug was weighed accurately in a glass stoppered flask. Added 100 ml of distilled water and shacked occasionally for 6 hours and then allowed to stand for 18 hours. Filtered rapidly taking care not to lose any solvent and pipetted out 25 ml of the filtrate in a pre-weighed 100 ml beaker and evaporated to dryness on a water bath. Kept in an air oven at 105ºC for 6 hours. Cooled in a desiccator and weighed. Repeated the experiment twice, and taken the average value. The percentage of water soluble extractive was calculated by the formula given below.

Calculation:

$$\text{Percentage of water soluble extract} = \frac{\text{Weight of the extract}}{\text{Weight of sample taken}} \times \frac{100}{25} \times 100$$

7. Alcohol-soluble extractive of the sample

4 g of the sample was weighed accurately in a glass stoppered flask. Added 100 ml of distilled alcohol (approximately 95%) and shaken occasionally for 6 hours and then allowed to stand for 18 hours. Filtered rapidly taking care not to lose any solvent and pipetted out 25 ml of the filtrate in a pre-weighed 100 ml beaker and evaporated to dryness on a water bath. Kept in an air oven at 105ºC for 6 hours and cooled in a desiccator and weighed. Repeated the experiment twice, and taken the average value. The percentage of alcohol soluble extractive was calculated by the formula given below

$$\text{Percentage of alcohol soluble extract} = \frac{\text{Weight of the extract}}{\text{Weight of sample taken}} \times \frac{100}{25} \times 100$$

The results of physicochemical properties were represented in Table no-3

5.3 Chemical analysis

The chemical analysis of *Gandhaga sarkkarai* was carried out in Biochemistry Lab, National Institute of Siddha.

S.No	EXPERIMENT	OBSERVATION	INFERENCE
1.	Physical Appearance of extract	Yellow in colour	_
2.	**Test for Silicate** a. A small amount of the sample was shaken well with distilled water. b. A small amount of the sample was shaken well with Conc. HCL/ Con. H_2SO_4	Sparingly soluble Completely soluble	Absence of Silicate
3.	**Action of Heat:** A small amount of the sample was taken in a dry test tube and heated gently at first and then strong.	No White fumes evolved. No brown fumes evolved.	Absence of Carbonate Absence of Nitrate.
4.	**Flame Test:** A small amount of the sample was made into a paste with con. HCl in a watch glass and introduced into non-luminous part of the Bunsen flame.	No bluish green flame appeared	Absence of copper
5.	**Ash Test:** A filter paper was soaked into a mixture of extract and dil. cobalt nitrate solution and introduced into the Bunsen flame and ignited.	No yellow colour flame appeared	Absence of sodium

Preparation of Extract:

5 gm of *Gandhaga Sarkkarai* was taken in a 250 ml clean beaker and added with 50ml of distilled water. Then it was boiled well for about 10 minutes. Then it was cooled and filtered in a 100 ml volumetric flask and made up to 100 ml with distilled water. This preparation was used for the qualitative analysis of acidic/basic radicals and chemical constituents in it.

S.No	EXPERIMENT	OBSERVATION	INFERENCE
	I. Test For Acid Radicals		
1.	**Test for Sulphate:** a.2 ml of the above prepared solution was taken in a test tube to this added 2 ml of 4% ammonium oxalate solution b. 2 ml of the above prepared solution was added with 2 ml of diluted HCL until the effervescence ceases off. Then 2 ml of Barium Chloride solution was added.	Cloudy appearance present White precipitate insoluble in con. HCL was obtained.	Presence of Sulphate Presence of Sulphate was confirmed
2.	**Test for Chloride:** 2 ml of the above prepared solution was added with 2 ml of dil- HNo_3 till the effervescence ceases. Then 2ml of silver nitrate was added.	No Cloudy appearance present.	Absence of Chloride
3.	**Test for Phosphate:** 2 ml of the solution was treated with 2 ml of ammonium molybdate solution and 2 ml of con.HNo3	Cloudy yellow appearance formed	Presence of Phosphate

4.	**Test for Carbonate:** 2 ml of the solution was treated with 2 ml diluted magnesium sulphate solution	No cloudy appearance	Absence of carbonate
5.	**Test for Nitrate:** 1 gm of the substance was heated with copper turning and concentrated H_2So_4 and viewed the test tube vertically down.	No Brown gas was evolved	Absence of nitrate
6.	**Test for Sulphide:** 1 gm of the substance was treated with 2 ml of con. HCL	No rotten egg smelling gas evolved	Absence of sulphide
7.	**Test for Fluoride & Oxalate:** 2 ml of extract was added with 2 ml of dil. Acetic acid and 2 ml calcium chloride solution and heated**.**	No cloudy appearance.	Absence of fluoride and oxalate
8.	**Test for Nitrite:** 3 drops of the solution was placed on a filter paper, on that-2 drops of acetic acid and 2 drops of Benzidine solution were placed.	No characteristic changes	Absence of nitrite
9.	**Test for Borate:** 2 Pinches (50mg) of the solution was made into paste by using sulphuric acid and alcohol (95%) and introduced into the blue flame.	Bluish green colour flame not appeared	Absence of borate

S.No	EXPERIMENT	OBSERVATION	INFERENCE
II. Test For Basic Radicals			
1.	**Test for Lead:** 2ml of the solution was added with 2ml of potassium iodine solution.	No Yellow precipitate appeared	Absence of lead
2.	**Test for Copper:** a. One pinch (25 mg) of solution was made into paste with con. HCl in a watch glass and introduced into the non-luminous part of the flame. b. 2ml of extract is added with excess of ammonia solution.	No blue colour flame appeared No blue colour precipitate appeared	Absence of copper Absence of copper
3.	**Test for Aluminium:** To the 2 ml of solution sodium hydroxide was added in drops to excess.	No characteristic changes	Absence of Aluminium.
4.	**Test for Iron:** a. To the 2 ml of solution add 2 ml of ammonium thiocyanate solution. b. To the 2ml of extract 2ml ammonium thiocyanate solution and 2ml of con HNo3 is added	No mild Red colour appeared. No Blood Red colour appeared	Absence of Iron
5.	**Test for Zinc:** To 2 ml of the solution sodium hydroxide solution was added in 5 drops to excess and ammonium chloride is added.	No White precipitate was formed	Absence of Zinc

6.	**Test for Calcium:** 2 ml of the solution was added with 2 ml of 4% ammonium oxalate solution	No Cloudy appearance and white precipitate was obtained	Absence of calcium
7.	**Test for Magnesium:** To 2 ml of solution sodium hydroxide solution was added in drops to excess.	No White precipitate was obtained	Absence of magnesium
8.	**Test for Ammonium:** To 2 ml of solution 1 ml of Nessler's reagent and excess of sodium hydroxide solution are added.	No Brown colour appeared	Absence of ammonium
9.	**Test for Potassium:** A pinch (25 mg) of solution was treated off with 2 ml of sodium nitrite solution and then treated with 2 ml of cobalt nitrate in 30% glacial acetic acid.	Yellow precipitate was obtained	Presence of potassium
10.	**Test for Sodium**: 2 pinches (50 mg) of the solution is made into paste by using HCl and introduced into the blue flame of Bunsen burner.	No yellow colour flame evolved	Absence of sodium
11.	**Test for Mercury**: 2 ml of the solution was treated with 2 ml of sodium hydroxide solution.	No Yellow precipitate obtained	Absence of Mercury

12.	**Test for Arsenic:** 2 ml of the solution was treated with 2 ml of sodium hydroxide solution.	No Brownish red precipitate obtained	Absence of arsenic

III. Miscellaneous			
1.	**Test For Starch:** 2ml of solution was treated with weak dil.Iodine solution	Blue colour developed	Presence of starch
2.	**Test For Reducing Sugar:** 5ml of Benedict's qualitative solution was taken in a test tube and allowed to boil for 2 minutes and added 8 to 10 drops of the extract and again boil it for 2 minutes. The colour changes were noted.	Brick red colour is developed	Presence of reducing sugar
3.	**Test For The Alkaloids**: a) 2ml of the solution was treated with 2ml of dil.potassium lodide solution. b) 2ml of the solution was treated with 2ml of dil.picric acid. c) 2ml of the solution was treated with 2ml of dil.phosphotungstic acid.	Yellow colour developed	Presence of Alkaloid
4	**Test For Tannic Acid:** 2ml of solution was treated with 2ml of dil. ferric chloride solution	No Blue-black precipitate was obtained	Absence of Tannic acid
5	**Test For Unsaturated Compound:** To the 2ml of solution, 2ml of dil. Potassium permanganate solution was added.	Potassium permanganate was not decolourised	Absence of unsaturated compound

6	**Test For Amino Acid:** 2 drops of the solution was placed on a filter paper and dried well. 20ml of Burette reagent was added**.**	No Violet colour appeared	Absence of amino acid
7.	**Test For Type of Compound:** 2ml of the solution was treated with 2 ml of dil. ferric chloride solution.	No green and red colour developed No Violet colour developed No Blue colour developed.	Absence of quinolepinephrinepyrocatechoantipyrine . Aliphatic amino acid and meconic acid. Apomorphine salicylate and Resorcinol were absent Morphine, Phenol cresol and hydrouinone were Absent.

Results

The results of acid and basic radicals were showed in Table no - 4 and Table no - 5.

5.5 Elemental analysis (ICP-OES)

Figure no - 9

The analysis of heavy metals and trace elements were estimated by using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). The Experimental Procedure was done at SAIF, IIT Madras, Chennai-36.

Icp-Oes Inductively Coupled Plasma Optical Emissions Spectrometry (ICP-OES)

ICP, abbreviation for Inductively Coupled Plasma, is one method of optical emission spectrometry. When plasma energy is given to an analysis sample from outside, the component elements (atoms) are excited. When the excited atoms return to low energy position, emission rays (spectrum rays) are released and the emission rays that correspond to the photon wavelength are measured. The element type is determined based on the position of the photon rays and the content of each element is determined based on the ray's intensity.

To generate plasma, first argon gas is supplied to torch coil and high frequency electric current is applied to the work coil at the tip of the torch tube. Using the electromagnetic field created in the torch tube by the high frequency current, argon gas is ionized and plasma is generated. This plasma has high electron density and temperature (10000k) and this energy is used in the excitation-emission of the sample. Solution samples are introduced into the plasma in an atomized state through the narrow tube in the center of the torch tube.

Sample preparation

1. Solids cannot be analyzed directly. Such samples should be made into clear aqueous medium quantitatively. When acids are used to prepare solutions, care should be taken. The concentration of the acids in the final provided solution should not be more than 2 % v/v. Highly acidic and organic solutions cannot be analyzed. As a guide line, weigh exactly around 200 mg of substance and dissolve it in 5 ml of 5% of water or aqua regia or whatever acid to make 100 ml of final solution. Make proper dilutions, if necessary, free HF should not present in the final solution to be aspirated.
2. Ideal concentration is around 100 ppm of the element of interest.
3. Total dissolved solids should be not more than 0.2% w/v in the final solution.
4. Very dilute solution may not give reliable results. Each element has a detection limit.
5. A minimum solution volume of 25 ml is necessary for analysis.
6. In ICP intensity of light emitted when the sample "sprayed or aspirated into an argon plasma" is measured at different wavelengths. The intensity of light at a given wavelength will be proportional to a particular elemental ion concentration. The intensity is calibrated with known standard concentration. For accurate quantitative results it is necessary to stimulate the sample matrix condition with that of the standard. Each element generally will have many emission lines and the sensitivity is different for each of this wave length. When more than one element is present it is quite common that some emission lines interfere due to overlapping.
7. It is preferable to use plastic containers for sample handling and preserving samples for ICP-OES analysis. Glass containers can give problems especially when analyzing certain metal ions at low concentration.

Result

The analytical result of heavy metals and trace elements in *Gandhaga Sarkkarai* using ICP-OES were showed in Table no- 6.

5.6 Scanning Electron Microscopy (SEM), Energy Dispersive X-ray Spectrometry (EDAX)

Figure no - 10

The particle size of the *Gandhaga Sarkkarai* was determined using High resolution scanning electron microscopy (HR SEM). The Experimental Procedure was done at Department of Materials science, Madurai Kamaraj University, Madurai 21.

A Scanning Electron Microscope (SEM) is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that contain information about the sample's surface topography and composition. The electron beam is scanned in a raster scan pattern, and the beam's position is combined with the detected signal to produce an image. It is a powerful and mature technique in the examination of materials, widely in metallurgy, geology, biology and medicine.

The Quanta 200 FEG Scanning Electron Microscope (SEM) is a versatile high resolution scanning electron microscope with three modes of operation namely,

1. High vacuum (HV) mode for metallic (electrically conducting) sample

2. Low vacuum (LV) mode for insulating, ceramic, polymeric (electrically insulating)
3. Environment scanning electron microscope (ESEM) for biological samples

Apart from giving the high resolution surface morphological images, the Quanta 200 FEG also has the analytical capabilities such as detecting the presence of elements down to boron on any solid conducting materials through the Energy Dispersive X-ray spectrometry (EDX) providing crystalline information from the few nanometer depth of the material surface via electron back scattered detection (BSD) system attached with microscope and advanced technological PBS (WDS) for elemental analysis. EDX analysis is useful in identifying materials and contaminants, as well as estimating their relative concentrations on the surface of the specimen. The EDX analysis system works as an integrated feature of a scanning electron microscope (SEM) and cannot operate on its own without the latter.

Principle

The primary electron beam interacts with the sample in a number of key ways: -

- Primary electrons generate low energy secondary electrons, which tend to emphasize the topographic nature of the specimen.
- Primary electrons can be backscattered which produces images with a high degree of atomic number (Z) contrast.
- Ionized atoms can relax by electron shell-to-shell transitions, which lead to either X-ray emission or Auger electron ejection.
- The X-ray emitted are characteristic of the elements in the top few μm of the sample and are measured by the EDX detector.

Method : A representative portion of each sample was sprinkled on to a double side carbon tape and mounted on aluminium stubs, in order to get a higher quality secondary electron image for SEM examination.

Resolution **:** 1.2 nm gold particle separation on a carbon substrate

Magnification : From a min of 12 X to greater than 1,00,000 X.

Application **:** To evaluate grain size, partical size distributions, material homogeneity and inter metallic distributions.

Sample required

- Any dimension (Height or Diameter) less than 10mm.
- The ideal shape of a sample was that of a button on a shirt. However, the other sizes can also be accommodated only after the discussion with the system operator.
- If the sample was not electrically conducting, it will require silver or gold coating.
- If the sample was a powder, make a normal button size pellet of the sample.
- If the sample was insulator (or) polymeric (or) electrically non-conducting it needs to be coated with carbon.

Sample preparation

- Sample preparation can be minimal or elaborate for SEM analysis, depending on the nature of the samples and the data required.
- Minimal preparation includes acquisition of a sample that will fit into the SEM chamber and some accommodation to prevent gorge build-up on electrically insulating samples.
- Most electrically insulating samples are coated with a thin layer of conducting material, commonly carbon, gold or some other metal or alloy. The choice of material for conductive coatings depends on the data to be acquired.
- Carbon is most desirable if elemental analysis is a priority, while metal coatings are most effective for high resolution electron imaging applications [57].

Calculation of the particle size:

The horizontal line in the right corner of the micrograph corresponds to micro in length would be given. A comparison could be made between the length of the particles visible in the micrograph with this line and the length of the particles was calculated.

Procedure:

An electron beam passing through an evacuated column is focused by electromagnetic lenses onto the specimen surface. Since an electron is a charged particle, it has a strong interaction with the specimen (due to coulomb interaction).So when an electron beam images on a specimen, it is scattered by atomic layers near the surface of the specimen. As a result, the direction of electron motion changes and its energy is partially lost. Once an incident electron (primary electron) enters a substance, its direction of motion is influenced by various obstructions (multiple scattering), and follows a complicated trajectory which is far from a

straight line. Also, when electrons with the same energy are incident on the specimen surface, a portion of electrons is reflected in the opposite direction (back scattered) and the remainder is absorbed by the specimen (exciting X- rays or other quanta in the process). If the specimen is sufficiently thin, the electron can pass all the way through the specimen (transmitted electrons, scattered or non-scattered).

The depth at which various signals are generated due to electron beam – specimen interaction indicates the diffusion area of the signals in the specimen in addition to the local chemistry of the specimen. Secondary electrons mainly indicate information about the surface of a specimen. Since secondary electrons do not diffuse much inside the specimen, they are most suitable for observing the fine-structures of the specimen surface. That is to say, sharp scanning images with high resolution can be expected from secondary electrons, because of the smaller influence on resolution by their diffusion.

As the incident electron energy increases, the probability of incident electrons Colliding with elemental components of the specimen and releasing secondary electrons also increases. In other words, as the incident energy increases, the emission of electrons from the specimen also increases. However, as the energy increases beyond a certain level, the incident electrons penetrate deeper into the specimen with the result that the specimen derived electrons use up most of their energy to reach the specimen surface. Consequently, the electron emission yield decreases. Therefore, the peak secondary electron emission yield occurs at a specific entry level of the incident electrons.

In order to verify the existence of a substance and recognize its shape, the image contrast must be well defined. In other words, even if a system boasts extremely high resolution, if image contrast is poor, it would be extremely difficult to determine the existence of a substance, let alone recognize its shape. Another important feature of the SEM is the three-dimensional appearance of the specimen image, which is a direct result of the large depth of field.

Applications:

The SEM is capable of examining objects at very low magnification. This feature is useful in viewing particle size and shape of any composition at various stages of preparation in *Siddha* system as well as other fields.The large depth of field available in the SEM makes it possible to observe 3-dimensional objects in stereo. Today, a majority of SEM facilities are equipped with X-ray analytical capabilities. Thus topographic crystallographic and compositional information can be obtained rapidly, efficiently and simultaneously from the same area.

The author was chosen this analysis for detecting Particle size of the classical *Siddha* herbo-mineral drug *Gandhaga sarkkarai* .

Advantages of SEM

1. It gives detailed 3D and topographical imaging and the versatile information garnered from different detectors.
2. This instrument works very fast
3. Modern SEMs allow for the generation o data in digital form
4. Most SEM samples require minimal preparation actions.

Disadvantages of SEM

1. SEMs are expensive and large
2. Special training is required to operate an SEM.
3. The preparation of samples can result in artifacts.
4. SEMs are limited to solid samples.
5. SEMs carry a small risk of radiation exposure associated with the electrons that scatter from beneath the sample surface.

Results

The results were represented in Table no -7.

5.7 Fourier Transform Infrared (FTIR)

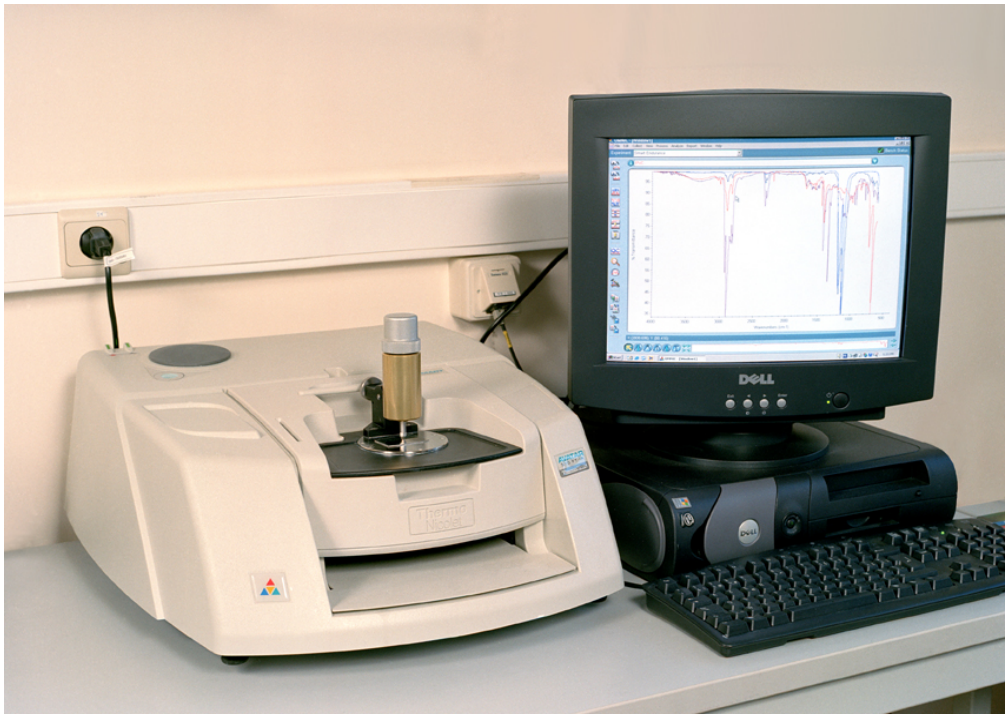

Figure no -11

The Fourier Transform Infrared Spectroscopy test was carried out for *Gandhaga Sarkkarai* as per the standard procedure. The Experimental Procedure was done SAIF, IIT Madras, Chennai 36.

Fourier Transform Infrared Spectroscopy is a powerful tool for identifying types of chemical bonds in a molecule by producing an infrared absorption spectrum that is like a molecular "fingerprint". This property is used for characterization of organic, inorganic and biological compounds. The band intensities are proportional to the concentration of the compound and hence qualitative estimations are possible. The IR spectroscopy is also carried out by using Fourier transform technique.

Description:

The Perkin Elmer Spectrum FTIR instrument consists of globar and mercury vapour lamp as sources, an interferometer chamber comprising of KBr and mad Mylar beam splitters followed by a sample chamber and detector. Entire region of 400-4500 cm-1 is covered by

this instrument. The spectrometer works under purged conditions. Solid samples are dispersed in KBr or polyethylene pellets depending on the region of interest. This instrument has a typical resolution of 1.0 cm -1 cm. Signal averaging, signal enhancement, base line correction and other spectral manipulations are possible.

The interference pattern obtained from a two beam interferometer as the path difference between the two beams is altered, when Fourier transformed, gives rise to the spectrum. The transformation of the interferogram into spectrum is carried out mathematically with a dedicated on- line computer.

Model : Spectrum 1 FTIR spectrometer

Scan range : MIR 450-4500 cm-1

Resolution : 1.0 cm-1

Sample required : 50 mg solid or liquid.

Sample preparation

Solid : KBr or Nujol mull method

Liquid : Cal / TIBr cells

Gas : Gas cells

KBr method:

The sample was grounded using an agate motor and pestle to give a very fine powder. The finely powder sample was mixed with about 100 mg dried potassium bromide salt. The mixture was then pressed under hydraulic press using a die to yield a transparent disc (measure about 13mm diameter and 0.3 mm in thickness) through which the beam of spectrometer passed.

Applications:

Infrared spectrum is useful in identifying the functional groups like – OH, -CN, -NH2, etc. Also quantitative estimation is possible in certain cases for chemical, pharmaceuticals, petroleum products etc. Resins from industries, water and rubber samples can be analysed. Blood and food materials can also be analysed.

Measurements Techniques:

The procedure for recording the %T or %A is as follows:

1. Air is first scanned for the reference and stored. The sample is then recorded andfinally the ratio of the sample and reference data is computed to give required %T or %A at various frequencies.
2. Study of substances with strong absorbance bands and weak absorbance bands aswell as possible.
3. Small amount of samples are sufficient
4. High resolution is obtained.

Procedure:

Typically,1.5 mg of protein, dissolved in the buffer used for its purification, were centrifuged in a 30 K Centric on micro concentrator (Amicon) at 3000_g at 4oC until a volume of approximately 40 Al.

1. Then, 300 Al of 20 mM buffer, prepared in H_20 or $2H_20$, pH or p2H 7.2, were added and the sample concentrated again. The p2H value corresponds to the pH
2. meter reading + 0.4. The concentration and dilution procedure was repeated several times in order to completely replace the original buffer with the This buffer.
3. The washings took 24 h, which is the time of contact of the protein with the 2H20
4. medium prior FT-IR analysis. In the last washing, the protein was concentrated to fine a volume of approximately 40 Al and used for the infrared measurements.
5. The concentrated protein sample was placed in CaF2 windows and a 6 Am tin spacer or a 25 Am Teflon spacer for the experiments in H20 or 2H20, respectively. FT-IR spectra were recorded by means of a Perkin-Elmer -Spectrum-1 FT-IR spectrometer using a deuteratedtriglycinesulfate detector.
6. At least 24 h before, and during data acquisition, the spectrometer were continuously purged with dry air at a dew point of 40^oC. Spectra of buffers and samples were acquired at 2 cm_1 resolution under the same scanning and temperature conditions. In

the thermal denaturation experiments, the temperature was raised in 5°C steps from 20 to 95°C.

7. Before spectrum acquisition, samples were maintained at the desired temperature for the time necessary for the stabilization of temperature inside the cell (6 min).Spectra were collected and processed using the SPECTRUM software from Perkin-Elmer. Correct subtraction of H_20 was judged to yield an approximately flat baseline at 1900-1400 cm_ 1, and subtraction of $2H_20$ was adjusted to the removal of the$2H_20$ bending absorption close to 1220 cm_ 1

KBr Method

1. The sample is grounded using an agate mortar and pestle to give a very fine powder.
2. The finely powder sample is then mixed with about 100mg dried KBr salt.
3. The mixture is then pressed under hydraulic press using a die to yield a transparent disc and measure about 13mm diameter and 0.3mm in thickness.

Nujol Mull Method:

1. The sample is ground using an agate mortar and pestle to give a very fine powder.
2. A small amount is then mixed with nujol oil to give a paste and this paste is thenapplied between two sodium chloride plates.
3. The plates are then placed in the instrument sample holder ready for scanning.

Liquids:

1. Viscous liquids can be smeared in the cell and directly measured.
2. For dilute solutions, liquid cells and variable path length cells are employed.

Applications:

Infrared spectrum is useful in identifying the functional groups like -OH, -CN, -CO, -CH, -NH2, etc. Also quantitative estimation is possible in certain cases for chemicals, pharmaceuticals, petroleum products, etc. Resins from industries, water and rubber samples can be analyzed.

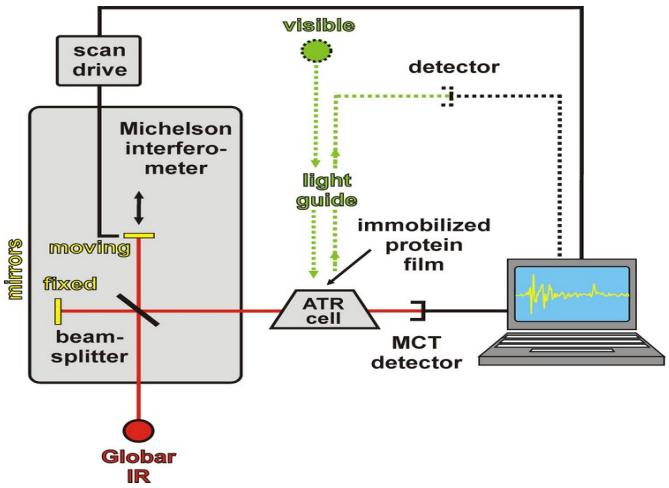

Fig.11.1-Mechanism of FTIR analysis

Analytical Capabilities:

1.Identifies chemical bond functional groups by the absorption of infrared radiation which excites vibrational modes in the bond.

2.Especially capable of identifying the chemical bonds of organic materials

3.Detects and identifies organic contaminants.

4.Identifies water, phosphates, sulphates, nitrates, nitrites, and ammonium ions

5.Detection limits vary greatly, but are sometimes $<10^{13}$ bonds/cm^3 or sometimes sub monolayer .Useful with solids, liquids, or gases.

Result

The result of FTIR was represented in Table no-8.

5.8 X- ray Diffraction

The X-ray powder diffraction test was carried out for *Gandhaga Sarkkarai* as per the standard procedure. The Experimental Procedure was done SAIF, IIT Madras, Chennai 36.

X-ray powder diffraction (XRD) is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. It is a compact advanced instrument. When X-rays falls over a crystal, it diffracts in a pattern characteristic to its structure. A diffraction pattern plots Intensity against the angle of detector. Diffraction occurs when light is scattered by a periodic array with the range of order, producing constructive interference at specific angles. The pattern contains information about the atomic arrangement in crystal. Amorphous materials like glass do not have periodic array with long range order, so they do not produce any significant diffraction pattern.

Sample required:

25 gm to be submitted

Sample preparation

1. Approximately 1gm was kept as a reference, 5gm was taken for sample preparation and the remainder was used for preparation of decalcified, fractioned 2- 20µ and less than 2µ samples.
2. Sample was disaggregated in waring blenders with 250ml hot distilled water until no lumps of sediment is visible.
3. The sample was centrifuged and the wash-water was decanted.
4. Then the sample was allowed to dry and disaggregated manually with a mortar and pestle.
5. Coarse grained sample was reduced to silt size.
6. Then it was placed in mortar and pestle grinders and heat generated grinding done under butanol for 2 hours.
7. After grinding, butanol as evaporated under heat lamps.
8. The ground sample was treated with trihexylamine acetate.
9. Then the sample was pressed into sample holder.

Benefits

It serves a major role in all stage of drug development, testing and production. It is an essential part of analytical research and development, quality control of the active ingredients, excipients and final products. It helps in elucidation of the relevant polymorphic and pseudo- polymorphic forms in pharmaceutical development.

Advantages

The PXRD analysis of crystalline compounds gives a diffraction pattern consisting of a well-defined, narrow, sharp and significant peak while amorphous materials do not give clear peaks rather the pattern has noise signals, smeared peak or it can have some short order bumps. Powder XRD is used to determine the crystallinity by comparing the integrated intensity of the background pattern to that of the sharp peaks.

Strengths

- Powerful and rapid (< 20 min) technique for identification of an unknown mineral.
- In most cases, it provides an unambiguous mineral determination.
- XRD units are widely available and minimal sample preparation is required.
- Data interpretation is relatively straight forward.

Limitations

- Homogeneous and single phase material is best for identification of an unknown.
- Must have access to a standard reference file of inorganic compounds (d-spacings, *hkl*s).
- Requires tenths of a gram of material which must be ground into a powder.
- For unit cell determinations, indexing of patterns for non-isometric crystal systems is complicated.
- Peak overlay may occur and worsens for high angle 'reflections'.

Result

The result of X-ray diffraction was represented in Figure 4.

5.9 Thermo-gravimetric analysis

The TGA test was carried out for *Gandhaga Sarkkarai* as per the standard procedure. The Experimental Procedure was done SAIF, IIT Madras, Chennai 36.

It is a method of thermal analysis in which the mass of a sample is measured over time as the temperature changes. This measurement provides information about physical phenomena, such as phase transitions, absorption and desorption as well as chemical phenomena including chemisorption, thermal decomposition and solid- gas reactions.

Principle

The principle of thermo-gravimetry is based on the simple fact that the sample is weighed continuously as it is being heated to elevated temperatures and changes in the mass of a sample are studied.

Changes in temperature affect the sample. Not all thermal changes/events bring a change in mass of sample *i.e.* melting, crystallization but some thermal events *i.e.* desorption, absorption, sublimation, vaporization, oxidation, reduction and decomposition bring a drastic change in mass of sample. It is used in analysis of volatile products, gaseous products lost during the reaction in thermoplastics, thermosets, elastomers, composites, films, fibres, coatings, paints, etc.

Types of Thermo-gravimetry

1.Isothermal/ Static Thermo-gravimetry

In this technique the sample weight is recorded as a function of time at constant temperature.

2. Quasistatic Thermo-gravimetry

In this technique the sample is heated to constant weight at each of the series of increasing temperature.

3. Dynamic Thermo-gravimetry

In this technique a sample is heated in an environment whose temperature is changing in predetermine manner generally at linear rate. Most of the studies are generally carried out with dynamic thermogravimetry. Therefore, it is generally referred to as thermogravimetry

Sample preparation

1. Sample preparation has a significant effect in obtaining good data.
2. It is suggested that maximizing the surface area of the sample in a TGA pan improves resolution and reproducibility of weight loss measurements.
3. Typically, 10-20mg of sample is preferred in most applications.
4. Whereas, if the sample has volatile 50-100mg of sample is considered adequate.
5. It is to be noted that most TGA instruments have baseline drift of ±0.025mg which is ±0.25% of a 10mg sample.
6. In heating rate method, Samples are heated at a rate of 10 or 20◦c/min in most cases. Lowering the heating rates is known to improve the resolution of overlapping weight losses.
7. In pure gas method, nitrogen is the most common gas used to purge samples in TGA due to its inert nature. Whereas, helium provides the best baseline.

Factors affecting TGA curve

1. Instrumental factors

a. Heating rate
b. Effect of furnace atmosphere
c. Sample holder

2. Sample characteristics

a. Weight of the sample
b. Sample particle size
c. Heat of reaction
d. Compactness of the sample
e. Previous history of the sample

Applications of TGA

1. From TGA, we can determine the purity and thermal stability of both primary and secondary standards.
2. Determination of the composition of complex mixture and decomposition of complex.
3. For studying the sublimation behavior of various substances.
4. TGA is used to study the kinetics of the reaction rate constant.
5. Used in the study of catalyst: The change in the chemical states of the catalyst may be studied by TGA techniques. Zinc- Zinc chromate is used as the catalyst in the synthesis of methanol.

Result

The result of Thermogravimetric analysis was represented in Figure 5.

5.10 Hptlc-Higher Perfomance Thin layer Chromotagraphy

High-perfomance thin layer chromatography (HPTLC) is an enhanced form of thin layer chromatography (TLC) .A number of enhancements can be made tp the basic method of thin-layer chromatography to automate the different steps , to increase the resolution achieved and to allow more accurate quantitative mesurements.

Automation is useful to overcome the uncertainly in droplet size and position when the sample is applied to the TLC plate by hand .One recent approach to automation has been the use of piezoelectric device and inkjet printers for applying the sample drugs .

The spot capacity can be increased by developing the plate with two different solvents, using two dimensional chromatography .The procedure begins with development of sample loaded plate with first solvent. After removing it , the plate is rotated 90 degree and developed with second solvent.

Thin layer chromatography (TLC) is a chromatographic technique used to separate the components of a mixture using a thin stationary phase supported by an inert backing. It may be performed on the analytical scale as a means of monitoring the progress of a reaction, or on the preparative scale to purify small amounts of a compound.

TLC/HPTLC is an analytical tool widely used because of its simplicity, relative low cost, high sensitivity, and speed of separation. TLC/HPTLC functions on the same principle as all chromatography: a compound will have different affinities for the mobile and stationary phases, and this affects the speed at which it migrates. The goal of TLC/HPTLC is to obtain well defined, well separated spots.

Retention Factor

After a separation is complete, individual compounds appear as spots separated vertically. Each spot has a retention factor (Rf) which is equal to the distance migrated over the total distance covered by the solvent.

$$Rf = \frac{\text{Distance travelled by sample}}{\text{Distance travelled by solvent}}$$

The Rf value can be used to identify compounds due to their uniqueness to each compound. When comparing two different compounds under the same conditions.

The compound with the larger Rf value is less polar because it does not stick to the stationary phase as long as the polar compound, which would have a lower Rf value.

Result

The result of HPTLC was represented in Figure no -17.

6.1 Anti-histamine activity

Anti-histamine activity of *Gandhaga Sarkkarai* on Wistar albino rats

Aim:

To evaluate the Anti-histamine activity of *Gandhaga Sarkkarai* in Wistar albino rats by Evans dye method.

Materials and methods:

Test Substance	**:**	*Gandhaga Sarkkarai.*
Animal Source	**:**	The Tamilnadu Veterinary and Animal Sciences University, Madhavaram.
Animals	**:**	Wistar albino rats (Male -12, Female -12)
Age	**:**	8-10 weeks.
Body Weight	**:**	250-250gm.
Acclimatization	**:**	14 days prior to dosing.
Veterinary examination	**:**	Prior and at the end of the acclimatization period.
Identification of animals	**:**	By cage number, animal number and individual marking by using Picric acid.
Diet	**:**	Pellet feed
Water	**:**	Aqua guard portable water in polypropylene bottles.
Housing & Environment	**:**	The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	**:**	24-28°C
Relative humidity	**:**	between 30% and 70%,
Air changes	**:**	10 to 15 per hour
Dark and light cycle	**:**	12**:**12 hours.

Selection of animals:

Healthy Wistar albino rats (150-200gm) of both sex were used for this study with the approval of the Institutional Animal Ethics Committee and obtained from the animal laboratory. **IAEC approved no: IAEC/LI/24/CLBMCP/2017**

The animals were kept in plastic cages and maintained at 24-28°C. All the rats were housed individually with free access to food, water and libitum. They were feed with standard diet and kept in well ventilated animal house they also maintained with alternative dark-light cycle of 12hrs throughout the studies. Rats were allowed an acclimatization period of 14 days before actual experiments. The rats were closely observed for any infection and if they show signs of infection they were excluded from the study. The animal experiment was performed with accordance legislation on welfare.

Experimental Design for Evan's dye method

The animals were divided into 4 groups. Each group has contains 6 animals .

Group 1 : Vehicle control (Honey)

Group II : Standard drug- Citrizine (20mg/kg)

Group III : Received test drug *Gandhaga Sarkkarai* (100mg/kg)

Group IV : Received test drug *Gandhaga sarkkarai* (200mg/kg)

Procedure : Vascular permeability test in rats :

Immediately after an i.v. injection of 1 ml of 1 % Evans blue in physiological saline, two sites on one side of the shaved back of animals were injected intradermally with 0.1 ml of physiological saline containing 0.1 µg histamine, Contralateral sites were injected intradermally with an equal volume of physiological saline (the control skin areas). *Gandhaga Sarkkarai* is given orally 30 min before to the injection of phlogistic agents. Thirty minutes later, the animals were sacrificed by overdose of anesthesia, and the skin was removed. Exudation of dye was calculated by subtracting the amount determined in the control skin area and expressed as the mean of two values obtained in each animal.

Result : The results of anti-histamine activity were showed in Table – 9 and Chart 3.

6.2 Anti- inflammatory activity

Anti-inflammatory activity of *Gandhaga sarkkarai* on Wistar albino rats

Aim

To evaluate the anti - inflammatory activity of *Gandhaga Sarkkarai* in Wistar albino rats by Carrageenan induced paw oedema method**.**

Materials and methods

Test Substance	**:**	*Gandhaga Sarkkarai.*
Animal Source	**:**	The Tamilnadu Veterinary and Animal Sciences University, Madhavaram.
Animals	**:**	Wistar albino rats (Male -12, female -12)
Age	**:**	6-8 weeks
Body Weight	**:**	150-200gm.
Acclimatization	**:**	14 days prior to dosing.
Veterinary examination	**:**	Prior and at the end of the acclimatization period.
Identification of animals	**:**	By cage number, animal number and individual marking by using Picric acid.
Diet	**:**	Pellet feed
Water	**:**	Aqua guard portable water in polypropylene bottles.
Housing & Environment	**:**	The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	**:**	24-28°C
Relative humidity	**:**	between 30% and 70%,
Air changes	**:**	10 to 15 per hour
Dark and light cycle	**:**	12**:**12 hours.

Selection of animals:

Healthy Wistar albino rats (150-200gm) of both sex were used for this study with the approval of the Institutional Animal Ethics Committee and obtained from the animal laboratory. **IAEC approved no: NIS/IAEC-III/02/29092016**

The animals were kept in plastic cages and maintained at 24-28°C. All the rats were housed individually with free access to food, water and libitum. They were feed with standard diet and kept in well ventilated animal house they also maintained with alternative dark-light cycle of 12hrs throughout the studies. Rats were allowed an acclimatization period of 14 days before actual experiments. The rats were closely observed for any infection and if they show signs of infection they were excluded from the study. The animal experiment was performed with accordance legislation on welfare.

Experimental Design for Carrageenan induced paw oedema method

The animals were divided into 4 groups. Each group has contain 6 animals .

Group 1 : Vehicle control (Honey)

Group II : Standard drug- Indomethacin (10mg/kg)

Group III : Received test drug *Gandhaga Sarkkarai* (100mg/kg)

Group IV : Received test drug *Gandhaga sarkkarai* (200mg/kg)

One hour after the administration of drugs, acute inflammation is produced by sub plantar injection of 0.1 ml of 1% suspension of carrageenan with normal saline in the right hind paw of the rats. Then the paw oedema is measured plethysomometrically at 0, 1, 2 and 3 hours after the carrageenan injection.

Result

The results of anti- inflammatory activity were showed in Table – 10 and Chart 4.

6 .3 Analgesic activity

Analgesic activity of *Gandhaga sarkkarai* on Swiss albino mice.

Aim:

To evaluate the Analgesic activity of *Gandhga sarkkarai* in Swiss albino mice by *Eddy's Hot plate method.*

Materials and methods:

Test Substance	**:**	*Gandhaga Sarkkarai.*
Animal Source	**:**	The Tamilnadu Veterinary and Animal Sciences University, Madhavaram
Animals	**:**	Swiss albino mice (Male -12, female -12)
Age	**:**	6-8 weeks
Body Weight	**:**	20-25gm.
Acclimatization	**:**	14 days prior to dosing.
Veterinary examination	**:**	Prior and at the end of the acclimatization period.
Identification of animals	**:**	By cage number, animal number and individual marking by using Picric acid.
Diet	**:**	Pellet feed
Water	**:**	Aqua guard portable water in polypropylene bottles.
Housing & Environment	**:**	The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	**:**	24-28°C
Relative humidity	**:**	between 30% and 70%,
Air changes	**:**	10 to 15 per hour
Dark and light cycle	**:**	12**:**12 hours.

Selection of Experimental animals:

The experimental protocol was submitted and approved by institutional Ethical Committee, **(IAEC approved no: NIS/IAEC-III/02/29092016)**. Swiss albino mice (20-25 gm) of approximate same age were employed in this investigation.

The animals were kept in plastic cages and maintained at 24-28°C. All the mice were housed individually with free access to food, water and libitum. They were feed with standard diet and kept in well ventilated animal house they also maintained with alternative dark-light cycle of 12hrs throughout the studies. Mice were allowed an acclimatization period of 14 days before actual experiments. The mice were closely observed for any infection and if they show signs of infection they were excluded from the study. The animal experiment was performed with accordance legislation on welfare.

Evaluation of Analgesic activity

Pain is the part of a defensive reaction against dysfunction of an organ or imbalance in its functions against potentially dangerous stimulus. The ascending pathway of pain includes the contralateral spinothalamic tract, lateral pons, mid brain to thalamus and ultimately through the somatosensory cortex of the brain that determines the locations, intensity and depth of pain

Eddy's Hot plate method:

Principle: Painful reactions can be produced in experimental animals by applying noxious stimuli such as thermal – using radiant heat as a source of pain, chemical – using irritants such as acetic acid and bradykinin and physical pressure – using tail compression.

The hot plate test was a test of the pain response in animals. It was used in basic pain research and in testing the effectiveness of analgesics by observing the reaction to pain caused by heat.

They used a behavioral model of nociception where behaviors such as jumping and hind paw-licking are elicited following a noxious thermal stimulus. Licking was a rapid response to painful thermal stimuli that was a direct indicator of nociceptive threshold. Jumping represents a more elaborated response, with a latency and encompasses an emotional component of escaping.

Animals

Mice 20-25 g were grouped in four groups, six animals in each group.

Grouping

Group I : Vehicle control

Group II : Standard drug - Pentazocine (10mg/kg/i.p)

Group III : *Gandhaga Sarkkarai* (100mg/kg)

Group IV : *Gandhaga Sarkkarai* (400mg/kg)

Equipment:

Eddy's Hot plate

Procedure:

Animals were weighed and placed on the hot plate. Temperature of the hot plate was maintained at 55 ± 1° C. Jumping response was seen. The time period (latency period), from when the animals were placed and until the responses occurred, were recorded using a stopwatch. To avoid tissue damage of the animals, 10 seconds was kept as a cut off time. The time obtained was considered the basal / normal reaction time in all the untreated groups of animals. Increase in the basal reaction time was the index of analgesia. All the animals were screened initially at least three times in this way and the animals showing a large range of variation in the basal reaction time were excluded from the study. A final reading of the basal reaction time was recorded for the included animals. After selecting the animals, the drugs were administered to all the groups at the stipulated doses. The reaction times of the animals were then noted at 0, 30, 60, 120 and 180 mins interval after drug administration.

Result

The results of analgesic activity were showed in Table – 11 and Chart 5.

7. Results

Many studies have been carried out to bring the efficacy and potency of the drug *Gandhaga Sarkkarai* . Traditional remedies are advantageous; it does suffer some limitations.

The main limitation is the lack of standardisation of raw materials, processing methods, the final products, dosage formulation, and the non- existence of criteria for quality control.

Standardization of the drug is more essential to derive the efficacy, potency of the drug by analysing it through various studies.

Following tables and charts are the results of physicochemical and phytochemical analysis. Physical characterization and estimation of basic and acidic radicals have been done and tabulated. Pharmacological activities of the drug were derived. Its result has been tabulated below.

5.1 ORGANOLEPTIC CHARACTERS

Table 1: Organoleptic characters of *Gandhaga sarkkarai*

1	**Colour**	**Yellow colour**
2	**Odour**	**Odourless**
3	**Texture**	**Fine powder**
4	**Taste**	**Tasteless**

5.2 PHYTOCHEMICAL ANALYSIS

Table 2: Phytochemical analysis of *Gandhaga Sarkkarai*

S.no	Phytochemicals	Test Name	H2O Extract
1.	Alkaloids	Mayer's Test	Negative
		Wagner's Test	Negative
		Dragendroff's Test	Negative
		Hager's Test	Negative
2.	Carbohydrates	Molisch's Test:	**Positive**
		Benedict's Test	**Positive**
3.	Glycoside	Modified Borntrager's Test	**Positive**
		Keller Killiani	Negative
4.	Saponin	Froth Test	**Positive**
		Foam Test	Negative
5.	Phytosterol	Salkowski's Test	Negative
6.	Phenols	Ferric Chloride Test	Negative
7.	Tannins	Gelatin Test	Negative
8.	Flavonoids	Alkaline Reagent Test	Negative
		Lead acetate Test	**Positive**
9.	Proteins and amino acids	Xanthoproteic Test	**Positive**
10.	Diterpenes	Copper Acetate Test	Negative
11.	Gum & Mucilage	Extract + Alcohol	Negative
12.	Fat & Fixed Oil	Spot Test	Negative
13.	Quinones	NAOH + Extract	**Positive**

Interpretation: Bioactive compounds are present in *Gandhaga Sarkkarai* .

---

*Phytochemical analysis was done at The T. N. Dr. M. G. R Medical university, Chennai-32

5.3 PHYSICOCHEMICAL ANALYSIS

Table 3: Physicochemical properties of *Gandhaga sarkkarai*

S.no	Parameters	Results
1.	pH	6.1
2	Loss on drying at 105ºC	1.42%
3.	Total Ash value	2.42%
4.	Acid insoluble ash	Less than 1%
5.	Water soluble ash	1.35%
6.	Water soluble extractive	6.32%
7	Alcohol soluble extractive	6.4%

Interpretation

In the physiochemical analysis, the pH of the drug *Gandhaga sarkkarai is 6.1*.It denotes that it is weakly alkaline in nature. So that , in the oral administration , the drug will get ionized in stomach and absorbed in intestine and send directly to the portal system.

The Total of volatile content and moisture present in the drug was established in loss of drying. Moisture content of the drug reveals the stability and its shelf-life .High moisture content can adversely affect the active ingredient of the drug.The low moisture content could get maximum stability and shelf-life.

The loss of drying value of *Gandhaga sarkkarai* was found to be 1.42% .hence the drug will not lose much of its volume on exposure to the atmospheric air at room temperature.

Ash constitutes the inorganic residues obtained after complete combustion of a drug. Thus Ash value is a validity parameter describe and to assess the degree of purity of a given drug.

Total ash value indicates the amount of minerals and earthy materials present in the plant material. The total inorganic content (potassium, sulphur etc.,) present in the drug is measured through the Total ash value and it is of 2.42 % for *Gandhaga Sarkkarai* ..

The acid insoluble ash value of the drug denotes the amount of siliceous matter present in the plant. The quality of the drug is better if the acid insoluble value is low. It is less than 1% for *Gandhaga sarkkarai.*

5.4 CHEMICAL ANALYSIS

Table 4: Results of acid radical studies of *Gandhaga sarkkarai*

S.no	Parameter	Observation	Result
1	Test for Sulphate	Cloudy appearance present. White precipitate insoluble in Con.HCL was obtained	**Positive**
2	Test for Chloride	-	Negative
3	Test For Phosphate	Yelow appearance present	**Positive**
4	Test For Carbonate	Cloudy yellow appearance present	Negative
5	Test For Nitrate	-	Negative
6	Test for Sulphide	No Rotten egg smelling gas evolved	Negative
7	Test For Fluride &oxalate	-	Negative
8	Test For Nitrite	-	Negative
9	Test For Borax	-	Negative

Interpretation

The acidic radicals test shows the presence of **Sulphate, Phosphate .**

***** The chemical analysis was carried out in Biochemistry Lab, NIS, Ch – 47

Table 5: Results of basic radicals studies of *Gandhaga sarkkarai*

S.no	Parameter	Observation	Result
1	Test for Lead	-	Negative
2	Test for Copper	-	Negative
3	Test For Aluminium	-	Negative
4	Test For Iron	-	Negative
5	Test For Zinc	-	Negative
6	Test for Calcium	-	Negative
7	Test For Magnesium	-	Negative
8	Test For Ammonium	-	Negative
9	Test For Potassium	Yellow precipitate was obtained	**Positive**
10	Test For Sodium	-	Negative
11	Test For Mercury	-	Negative
12	Test For Arsenic	-	Negative

Interpretation

The basic radical test shows the presence of **Potassium** and absence of heavy metals such as lead, arsenic and mercury.

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* The chemical analysis was carried out in Biochemistry Lab, National Institute Of Siddha.

5.5 ELEMENTAL ANALYSIS

Table 6: ICP-OES study results of *Gandhaga sarkkarai*

S. No	Elements	Wavelength in nm	mg/L
1	Aluminium	Al 396.152	BDL
2	Arsenic	As 188.979	BDL
3	Calcium	Ca 315.807	BDL
4	Cadmium	Cd 228.802	BDL
5	Copper	Cu 327.393	BDL
6	Iron	Fe 238.204	01.761 mg/L
7	Mercury	Hg 253.652	BDL
8	Potassium	K 766.491	03.801 mg/L
9	Magnesium	Mg 285.213	01.984 mg/L
10	Sodium	Na 589.592	04.320 mg/L
11	Nickel	Ni 231.604	BDL
12	Lead	Pb 220.353	BDL
13	Phosphorus	P 213.617	116.341 mg/L
14	Sulphur	S 180.731	**401.204 mg/L**
15	Zinc	Zn 206.200	BDL

BDL -Below Detection Limit , PPM - Parts per million .

Interpretation: The heavy metals were found to be within normal limits. The presence of other elements shows the therapeutic value of *Gandhaga sarkkarai* ..

5.6 ANALYSIS OF PARTICLE SIZE AND EDAX *

a) Scanning Electron Microscope (SEM)

GANDHAGA SARKKARAI – SEM With EDAX

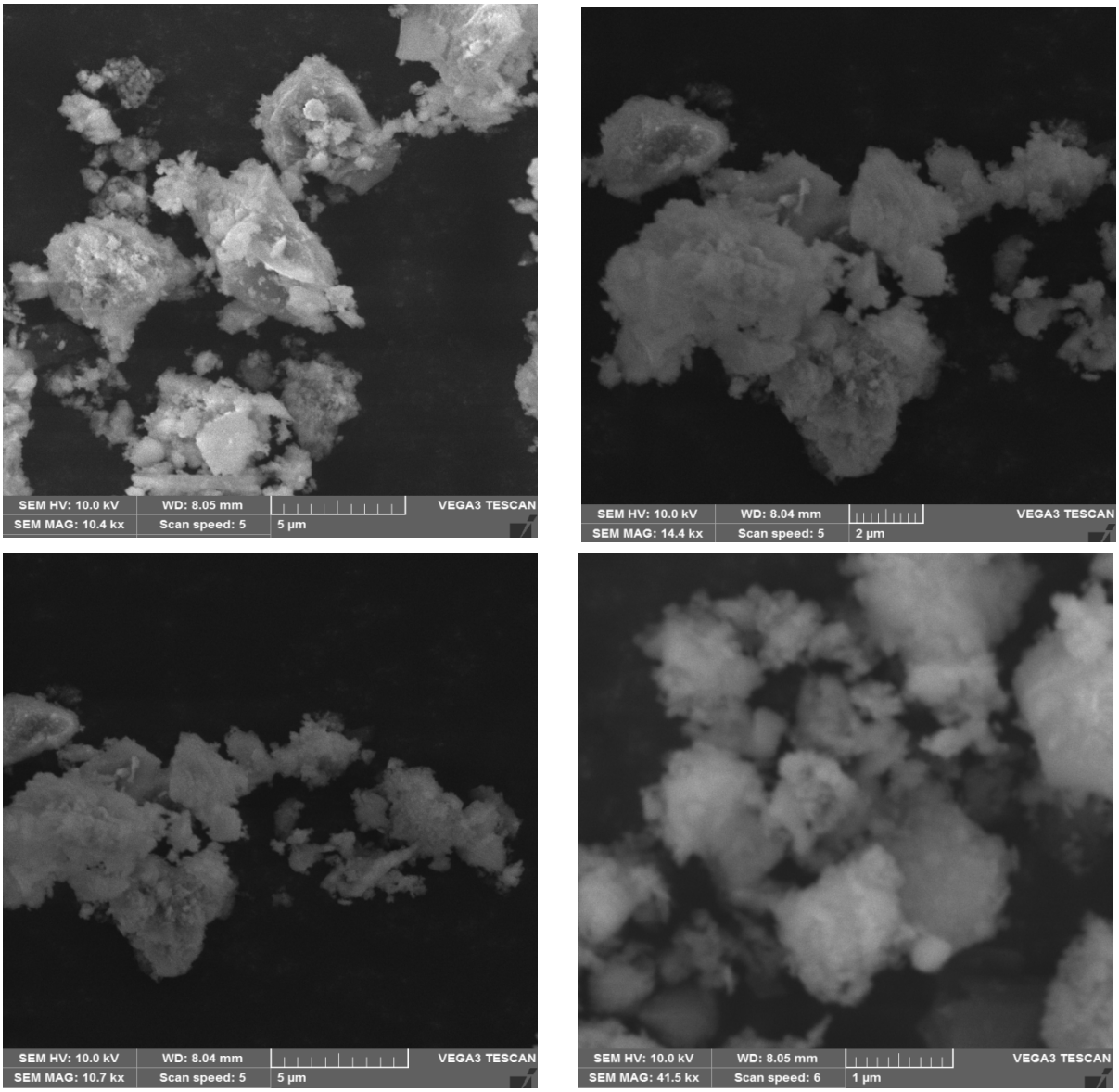


Figure 12 : SEM (Scanning Electron Microscope) image of Gandhaga sarkkarai sample

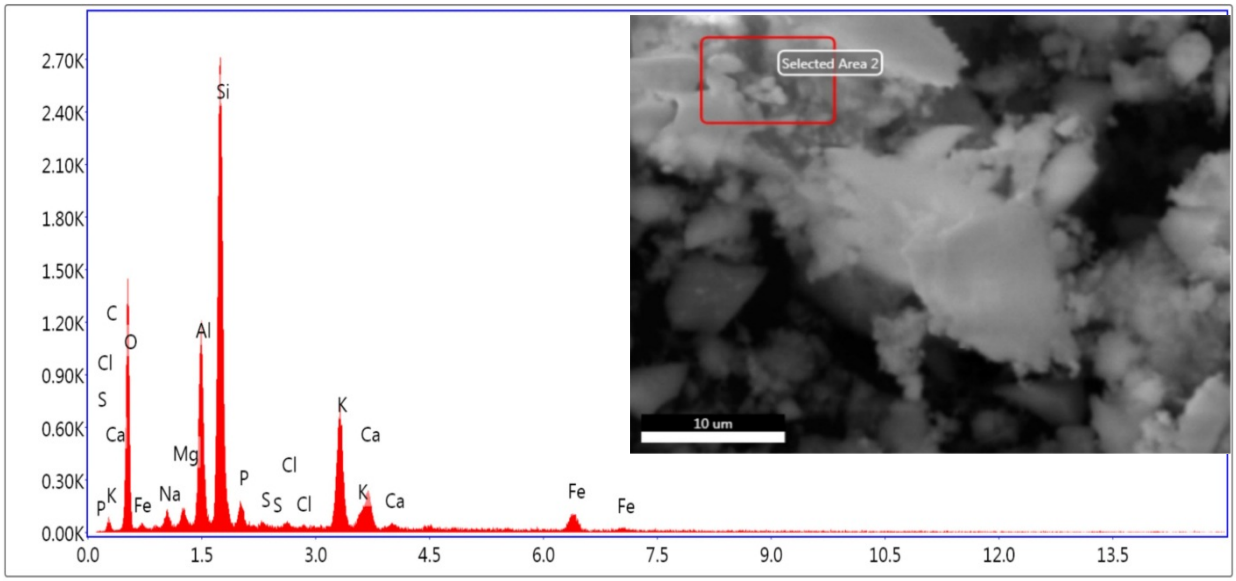


Figure 13 : EDAX analysis of Gandhaga Sarkkarai sample

Table – 7 :Edax analysis of Elements in Gandhaga Sarkkarai ,.

Element	Weight %	Atomic %	Net Int.	Error %
C K	5.95	9.89	31.28	20.29
O K	47.64	59.49	899.79	9.92
NaK	1.75	1.52	66.27	16.34
MgK	0.83	0.68	62.76	18.48
AlK	8.92	6.61	923.05	6.79
SiK	20.76	14.77	2348.82	5.62
P K	1.50	0.97	122.88	13.59
S K	0.14	0.09	14.21	64.59
ClK	0.11	0.06	12.16	64.02
K K	7.18	3.67	779.17	4.40
CaK	2.78	1.39	260.52	9.40
FeK	2.43	0.87	144.08	9.43

Description:

The VEGA TESCAN 3 instrument is used with tungsten filament to image the samples. The SEM imaging of the *Gandhaga Sarkkarai* sample shows that the particles are small and in the range of less than 100 nm as shown in Fig. 1. The particles are aggregate and individual particles are seen on the top of the clusters. The particle size is low because of the grounding for more than 6 hours for 4 days.

EDAX analysis shows the elements present in the sample as shown in Fig. 2. The table represents the weight and atomic percentage of sample. The presence of silicon Si , aluminum Aland Iron Fe is 23% is due to the usage of Kaiyanthangari charu and Venniramana Vengaya charu. The presence Calcium, Potassium, sodium and chlorine is minimum and may be from Nellikai Gandhagam juice. The carbon and oxygen is presence due to the calcination process.

5.7 FOURIER TRANSFORM INFRA-RED

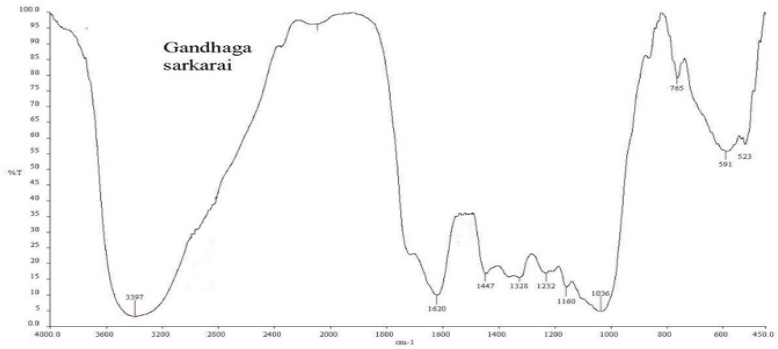


Figure no -14

Table 8: Vibrational modes and functional group of *Gandhaga sarkkarai* in FTIR

Wave number (cm-1)	Vibrational modes of *Gandhaga Sarkkarai* in IR region	Functional group
3397	O-H Strong, broad	Alcohol
1620	C=C Variable	Alkene
1447	C=C Medium-weak	Aromatic
1328	C-N Medium-weak	Amine
1232	C-O Strong	Ether
1160	C-O Strong	Ester
1036	C-F Strong	Alkyl halide
765	C-Cl Strong	Alkyl halide
591	C-Br Stretch	Alkyl halide

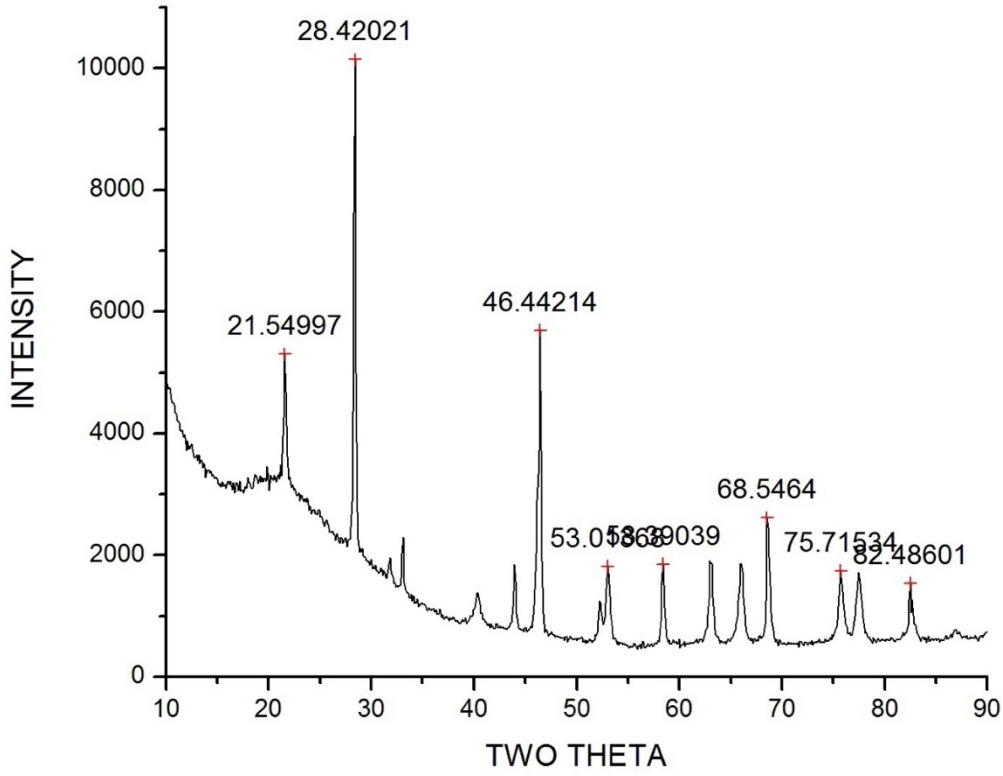
Interpretation

In the FTIR Spectra analysis, *Gandhaga sarkkarai* sample exhibits the peak value is 3397,1620,1441,1328,1232,1160,1036,765,591 shown in Table 8, at the wave number of having O-H Strong, C=C variable, C=C, C-O medium-weak, C-N medium-weak, C-O strong, C-O Stretch, C-F strong, C-Cl Strong, C-Br Strong.

This indicates the presence of some organic functional groups such as Alcohol , Alkene, Aromatic, Amine, Ether, Ester and Alkyl halide.

5.8 X-RAY DIFFRACTION

Figure no - 15:



Result Analysis of XRD pattern of Sample *Gandhaga Sarkkarai*

Observation

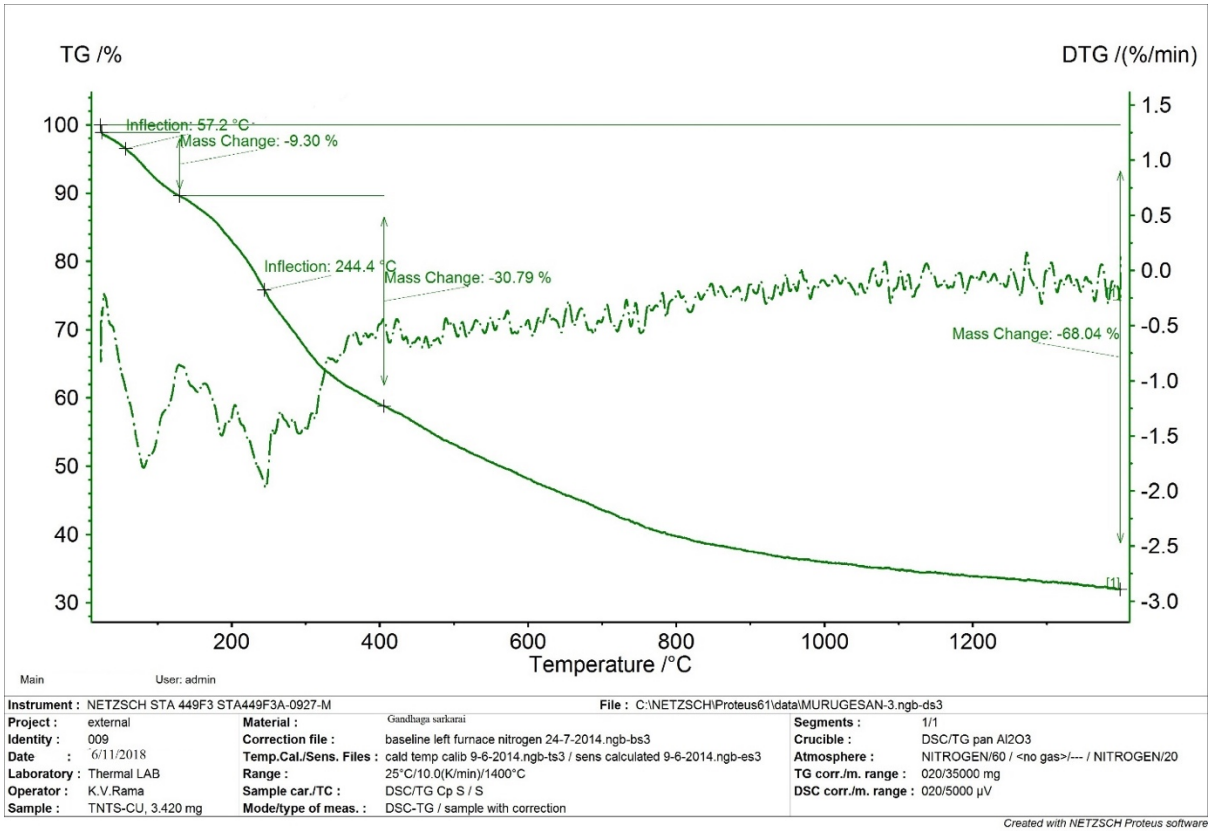
- The X-ray diffraction pattern of the prepared sample *Gandhaga Sarkkarai* reveals the presence of major peak with 2- Theta value of 28.42 with the intensity of 11000.
- Major peaks observed in test sample with 2-theta values of 28.42 and their corresponding intensities matching with the material sulphur.

Conclusion

- Further form this observation it was clouded that sulphur may the key ingredient in the sample *Gandhaga Sarkarai*.

5.9 THERMOGRAVIMETRIC ANALYSIS

Figure no - 16:



TGA Result Analysis of *Gandhaga Sarkarai*

- Thermo gravimetric analysis of *Gandhaga Sarkkarai* carried out at the maximum of 1300 degree centigrade. The main objective of the study is to evaluate the decomposition and stability limit of the prepared formulation *Gandhaga Sarkkarai.*
- Prepared formulation *Gandhaga Sarkkarai* seems to be stable at the temperature varying from 57 ºC to 400 ºC.
- Point of decomposition begins when the temperature increases beyond 400 ºC.
- Weight of the final residual matter was observed with 68.04% of residual volume.

Conclusion

From the result of the present investigation it was concluded that the formulation *Gandhaga Sarkkarai* seems to be stable at varying temperature ranges from 50 to 400 ºC.

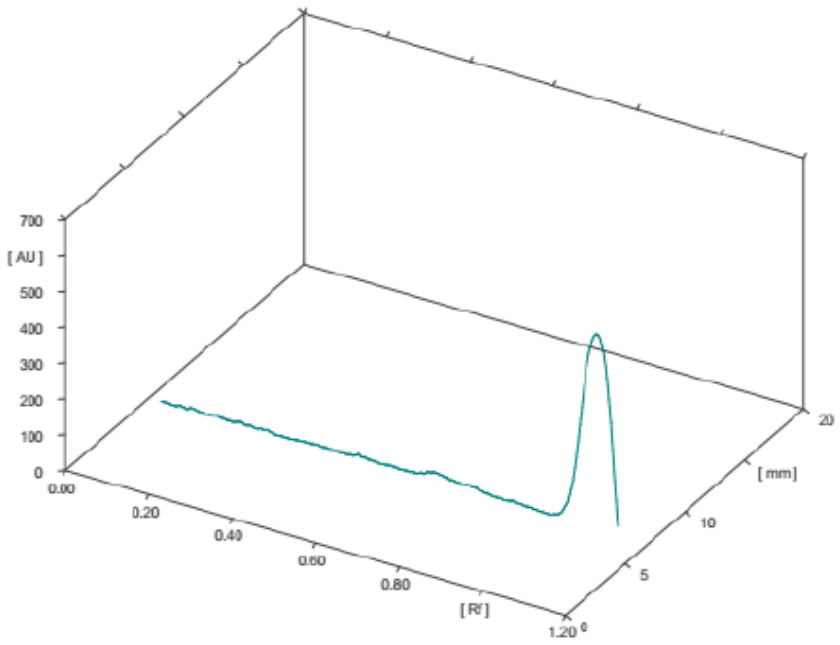
5.10 HPTLC-TLC (HIGHER PERFOMANCE THIN LAYER CHROMOTOGRAPHY)

Description of the Sample	Solid
Method of Analysis	
Instrument	CAMAG TLC SCANNER III
TLC Plate	Aluminium Coated Silica Gel – Merck
Mobile Phase	Toulene: Ethyl Acetate: Acetic Acid (1.5:1:0.5)
Extraction Solvent	Acetone

The sample (alcohol extract - 2µl) was applied in TLC aluminium sheet silica gel 60 F 254 (E. MERCK) and plate was developed using the solvent system Toluene: Ethyl acetate: Formic acid (8.5: 1.5: 0.2). After development the plate was allowed to dry in air and examined under UV – 254 nm, 366 nm and Visible light (Vanillin –Sulphuric acid).

TLC Analysis at 254 nm

TLC Analysis at 366 nm



HPTLC finger printing of Sample GS

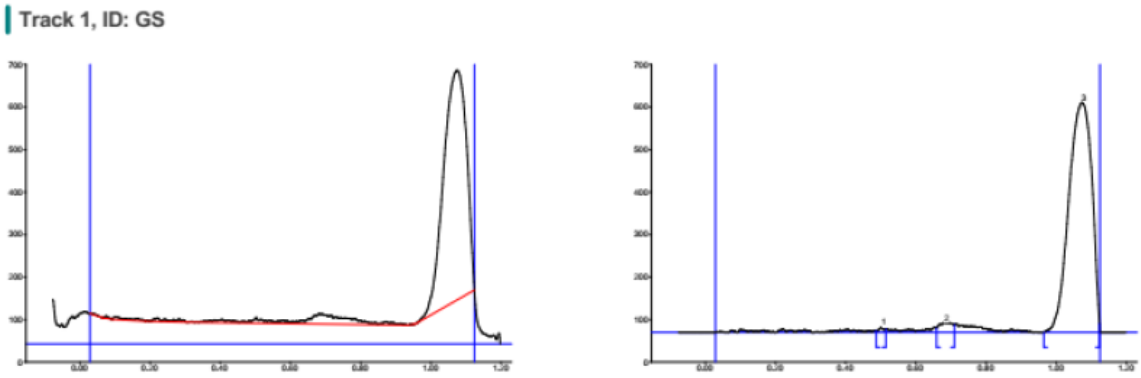


Figure no - 17

Peak Table

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.49	3.8	0.50	13.0	2.26	0.52	8.4	146.9	0.53
2	0.66	11.0	0.68	24.0	4.15	0.71	18.4	683.2	2.48
3	0.97	0.2	1.08	540.6	93.60	1.12	20.3	26729.4	96.99

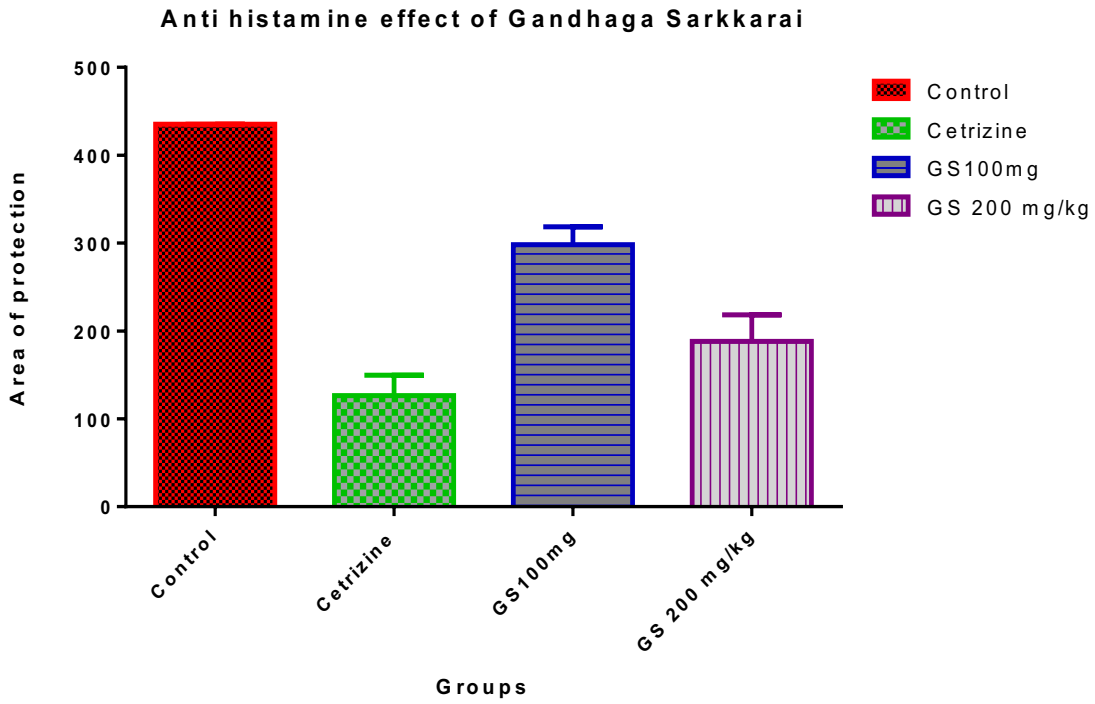
REPORT

HPTLC finger printing analysis of the sample *Gandhaga Sarkkarai* reveals the presence of three prominent peaks corresponds to presence of three versatile phyto-components present with in it. Rf value of the peaks ranges from 0.49 to 0.97. Further the peak 3 occupies the major percentage of area of 96.99 % which denotes the abundant existence of such compound. Followed by this peak 2 and 3 occupies the percentage area of 2.48 and 0.53 % .

6.1 ANTI-HISTAMINE ACTIVITY

Table 9: **Anti-histamine** activity of *Gandhaga sarkkarai* by Evan's dye method

S.no	Grouping	Area of protection from exudation of Dye in mm
1	Control	435.12±0.22
2	Cetirizine(STD) 10mg	126.43±0.07
3	Gandhaga Sarkkarai 100mg	298.14±0.14
4	Gandhaga Sarkkarai 200mg	188.12±0.15



N= 6, Values are expressed as mean ± SD, analysis was done by using ANOVA followed by Dunnett's Test. Test for significance is *P < 0.05, **P < 0.01, ***P < 0.001.

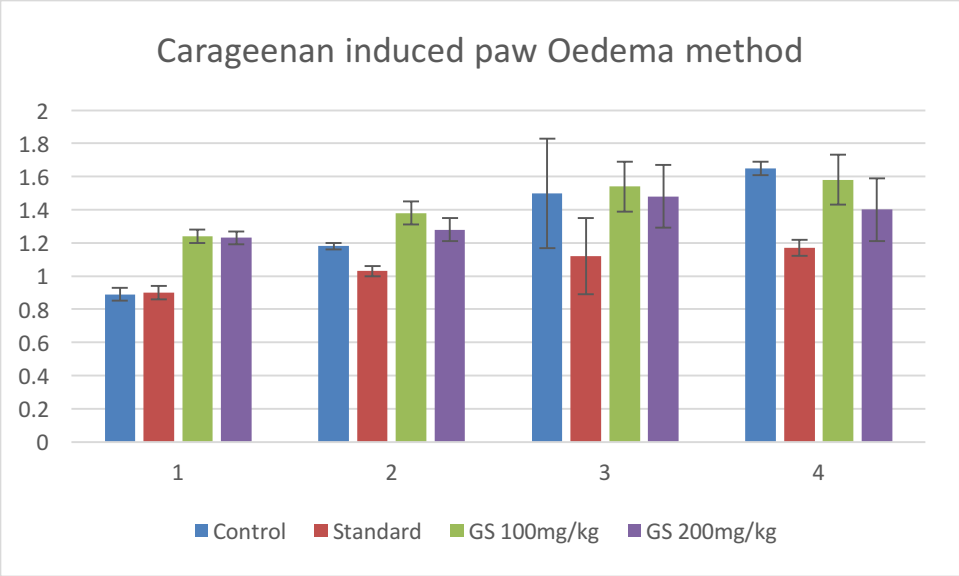
6.2 ANTI- INFLAMMATORY ACTIVITY

Table 10: Effect of *Gandhaga sarkkarai* on carrageenan induced paw edema method

Treatment	Percentage of inflammation after carrageenan injection at hr			
	0hr	1hr	2hr	3hr
Control	0.89±0.48	1.18±0.42	1.5±0.45	1.65±0.45
Indomethacin 10mg/kg	0.90±0.21	1.03±0.03***	1.12±0.07***	1.17±0.07***
GS 100mg/kg	1.23±0.33	1.38±0.23*	1.54±0.15*	1.58±0.19*
GS 200mg/kg	1.23±0.48	1.28±0.50**	1.48±0.38***	1.40±0.46***

N= 6, Values are expressed as mean ± SD, analysis was done by using ANOVA followed by Dunnett's method. Test for significance is *P < 0.05, **P < 0.01, ***P < 0.001.

Chart 4: Anti-inflammatory activity of *Gandhaga sarkkarai* by carrageenan induced paw edema method :



Result of Anti-inflammatory activity :

Gandhaga sarkkarai at 100 mg/kg dose showed significant anti-inflammatory activity ($P < 0.05$) at 1st hour when compared to control rats. At 200 mg/kg the drug showed significant ($P<0.01$) at 1st hour and ($P < 0.001$) at 2rd hour. Among the two doses of *Gandhaga sarkkarai*, 200 mg/kg have shown better anti- inflammatory activity ($P < 0.001$) when compared with control rats.

Conclusion

Thus it was concluded that administration of *Gandhaga sarkkarai* at the dose of 360 mg/kg exhibited significant ($p<0.001$) anti-inflammatory activity in Wistar albino rats when compared with control.

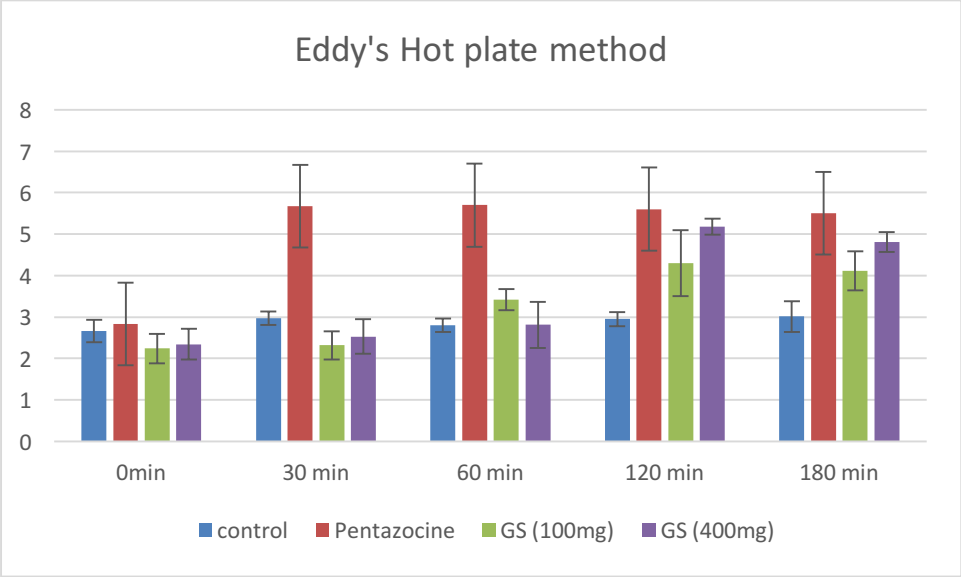
6.3 ANALGESIC ACTIVITY

Table 11: Analgesic activity of *Gandhaga sarkkarai* in Swiss albino mice

Groups	Treatment	Reaction time in sec				
		0min	30min	60min	120min	180min
I	Control	2.66±0.27	2.97±.16	2.8±0.16	2.95±0.17	3.01±0.37
II	Pentazocine (10mg/kg)	2.83±0.98	5.67±0.60***	5.7±0.66***	5.6±0.62***	5.5±0.6***
III	Low dose GS (100mg/kg)	2.24±0.35*	2.32±0.34*	3.42±0.25*	4.36±0.79*	4.11±0.47**
IV	High dose GS (400mg/kg)	2.34±0.37*	2.53±0.42*	2.81±0.55**	5.18±0.19***	4.81±0.24***

N= 6, Values are expressed as mean ± SD, analysis was done by using ANOVA followed by Dunnett's method. Test for significance is *P < 0.05, **P < 0.01, ***P < 0.001.

Chart 5: Analgesic activity of *Gandhaga sarkkarai* by Eddy's hot plate method



Result of Analgesic activity of *Gandhaga sarkkarai* :

Analgesic activity was carried out by Eddy's Hot plate method. *Gandhaga sarkkarai* at doses 100 mg/kg showed analgesic activity with the statistical significance of ($P<0.05$) at 60 min and ($P<0.01$) at 180 min when compared to control mice. At 26 mg/kg, the drug showed analgesic activity with significance ($P<0.01$) at 60 min and ($P < 0.001$) at 90 mins. Among the two doses of *Gandhaga sarkkarai* , 400 mg/kg dose have shown better analgesic activity ($P<0.01$) and ($P < 0.001$) when compared with control mice.

Conclusion

Thus it was concluded that administration of *Gandhaga sarkkarai* at the dose of 400 mg/kg exhibited significant ($p<0.001$) analgesic activity in Swiss albino mice when compared with control.

8. Discussion

The drug ***Gandhaga Sarkkarai*** was selected from the *Siddha* literature ***Anuboga Vaithiya Navaneetham,*** (part -VI, page no:46,47. preparation no:793) for Standardization and evaluation of pharmacological activities (Anti-histamine, Anti-inflammatory and Analgesic) in animal models.

The ingredients of the test drug were identified and authenticated .

The drug was prepared as per the procedure and subjected to various studies such as qualitative, quantitative and pharmacological activities.

Qualitative analysis includes Chemical analysis and Physicochemical properties of *Gandhaga sarkkarai*.

Quantitative analysis included ICP-OES, HR SEM with EDAX, FTIR, TGA, XRD analysis to reveal its potency and effectiveness against the disease. Also Anti-histamine activity, Analgesic activity and Anti-inflammatory activities were carried out in Animals .

Literature review include drug review, which consist of both Botanical aspect, Gunapadam aspect, Pharmacological review and Pharmaceutical aspect that supported the study.

In Chemical analysis, the drug *Gandhagam* revealed the presence of Sulphate, Sulphur, Potassium .These chemical elements present in *Gandhaga sarkkarai* enhances the Anti-histamine activity of the drug.

In Physicochemical analysis**,** the pH of the drug was 6.1. It denotes it is weakly acidic. Hence, in the oral administration of the drug it may indicate that drug will get ionized in stomach and will be absorbed in intestine and directly sent to portal system.

The loss on drying value of *Gandhaga sarkkarai* at 105^0C was found to be less than 1.42 %, hence the drug will not lose much of its volume on exposure to the atmospheric air at room temperature . High moisture content can adversely affect the active ingredient of the drug. Thus low moisture content could get maximum stability and better shelf life.

Ash value was found to be 2.42 %, it is the residue remaining after incineration that determines the inorganic substances present in the drug. Similarly, it can also detect the nature of the material, whether it is adulterate or not. Hence, determination of the ash value provides an idea for judging the identity and purity of the drug.

The acid insoluble ash value of the drug denotes the amount of siliceous matter present in the plant. The quality of the drug is better if the acid insoluble value is low. It is less than 1% for *Gandhaga sarkkarai.*

The *ICP-OES* results showed the heavy metals like As, Pb, Cd and Hg were found below detection limit in *Gandhaga sarkkarai.* Hence, it may be safe for human consumption. The presence of Sulphur 401.204 mg/L, Phosphorous 116.341mg/L were observed in major quantities. Iron, sodium, magnesium, potassium were observed at the level of 01.761 mg/L , 04.320 mg/L, 01.984 mg/A, 03.801 mg/L respectively are in a permissible amount in the *Gandhaga sarkkarai.* The drug *Gandhaga sarkkarai* which is rich in Sulphur and phosphorous explains that this drug may help in the treatment of Syphilis.

In *HR SEM* analysis, the sample shows that the particles are small and are less than 100 nm . The particles are aggregate and individual particles are seen on the top of the clusters. The particle size is low because of the grounding for more than 12 hours. This ensures the absorption of the drug was more active and the drug have increased bio-availability.

In *EDAX* analysis shows the presence silicon , sulphur , potassium , calcium. The presence of sulphur S , silicon Si aluminum Al and Iron Fe is 23% is due to the usage of Kaiyanthangari charu and Venniramana Vengaya charu.

FT-IR analysis indicates the presence of some organic functional groups such *Alcohol, Alkene, Aromatic, Ether, Ester and Alkyl halide*.

In *XRD* - The X-ray diffraction pattern of the prepared sample *Gandhaga Sarkkarai* reveals the presence of major peak with 2- Theta value of *28.42 with the intensity of 11000*.

Major peaks observed in test sample with 2-theta values of 28.42 and their corresponding intensities matching with the material sulphur .

In *TGA* The formulation *Gandhaga Sarkkarai* seems to be stable at varying temperature ranges from 50 to 400 ºC.

In *HPTLC* - Rf value of the peaks ranges from *0.49 to 0.97*.

For ***Anti-histamine activity*** Wistar albino rats of either sex were divided into 4 groups of 6 animals each. Group I received Vehicle control (Honey), Group II received Standard drug - Citrizine (10mg/kg), Group III and Group IV received *Gandhaga sarkkarai* at doses (100mg/kg) and (200 mg/kg) respectively. From the results it was concluded that administration of *Gandhaga sarkkarai* at the dose of 200 mg/kg exhibited significant ($p<0.001$) Anti-histamine activity in Wistar albino rats when compared with control .

For ***Anti- inflammatory activity*** Wistar albino rats of either sex were divided into 4 groups of 6 animals each. Group I received Vehicle control (Honey), Group II received Standard drug- Indomethacin (10mg/kg), Group III and Group IV received *Gandhaga sarkkarai* at doses (100mg/kg) and (200mg/kg) respectively. From the results it was concluded that administration of *Gandhaga sarkkarai* at the dose of 200 mg/kg exhibited significant ($p<0.001$) anti- inflammatory activity in Wistar albino rats when compared with control.

For ***Analgesic activity*** Swiss albino mice of either sex were divided into 4 groups of 6 animals each. Group I received Vehicle control (Honey), Group II received Standard drug- Pentazocine (10mg/kg, i.p.), Group III and Group IV received *Gandhaga sarkkarai* at doses (100mg/kg) and (400mg/kg) respectively. From the results it was concluded that administration of *Gandhaga sarkkarai* at the dose of 400 mg/kg exhibited significant ($p<0.001$) analgesic activity in Swiss albino mice when compared with control.

Thus by scrutinizing all the above mentioned factors it was concluded that the test drug *Gandhaga sarkkarai* has been scientifically validated and it was a safe and a potent Anti-histamine drug. It also possesses rich Anti- inflammatory and Analgesic activity which supports the effective treatment for managing for *Megam (syphilis), Premegham , Kiranthi , Purai (Whole Abscess) , Kai kaal kudaichal (Joint pain).*

9. Summary

- The test drug *Gandhaga sarkkarai* was selected from the *Siddha* literature *Anuboga Vaithiya Navaneetham* to evaluate the Anti-histamine, Anti-inflammatory and Analgesic activities.

- The dissertation started with an introduction explaining about the *Siddha* concept, prevalence of Syphilis and role of the test drug in treating Syphilis.

- All the ingredients were identified and authenticated by the experts and were purified and the medicine was prepared as mentioned in the *Siddha* literature.

- Review of literature in various categories were carried out. Siddha aspect, botanical aspect, mineralogical aspect and disclosed about the ingredients of the drug and strongly supported that it possesses Anti-histamine, Anti-inflammatory and Analgesic activities.

- The drug was subjected to qualitative analysis such as physicochemical, phytochemical, bio-chemical analysis. Quantitatively with FTIR, ICP-OES, HR-SEM with EDAX , HPTLC and TGA analysis which provided the key ingredients present in the drug thus it accounts the efficacy of the drug.

- Pharmacological studies revealed that the drug *Gandhaga sarkkarai* exhibited significant Anti-histamine , Anti-inflammatory and Analgesic activity in animal models.

- Results and discussion gives the necessary justifications to prove the potency of the drug.

- Conclusion gives a compiled form of the study and explains the synergistic effect of all the key ingredients and activities that supports the study.

10. Conclusion

From the above analytical studies (i.e qualitative and quantitative analysis) and the pharmacological activities (i.e Anti-histamine, Anti- inflammatory and Analgesic activities),this study is concluded that the study drug *Gandhaga sarkkarai* possesses a good Anti-histamine, Anti- inflammatory and Analgesic activities.Finally concluded that the study drug *Gandhaga sarkkarai* can be used for effective treatment of managing diseases like *Megam* (Syphilis) *, Kiranthi* , *Purai* (Whole abscess).

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